Nitrogen fixation by leguminous plants under landfill conditions

by

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A thesis submitted for the degree of Doctor of Philosophy in the University of Durham, England

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This thesis is entirely the result my own work. It has not been accepted for any other degree and is not being submitted for any diploma.

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ABSTRACT

The role and importance of rhizobia-legume symbiotic N_2 fixation under subtropical landfill conditions was studied by field survey, laboratory experiments and by plant trials on landfill sites in Hong Kong.

The field survey conducted on 13 landfill sites indicated that completed or on-going sites were generally bare of trees; only 25.8% of vegetated land was covered by trees. Legumes were more abundant (relative tree cover = 65.4%), compared with non-legumes (34.6%). Ten species of legume were found, two being especially abundant: Acacia confusa (55.1%), Leucaena leucocephala (5.3%).

Nodular 1-h acetylene reduction activity (ARA) of the two legumes was measured at different concentrations of O_2, CO_2 and CH_4. Maximum ARA was found at 20% O_2 and the ARA decreased as the O_2 decreased in the range of 16 - 1%. The ARA of Acacia confusa nodules was significantly inhibited at 30 - 50% CO_2 but the ARA of Leucaena leucocephala nodules was not significantly inhibited at the same concentrations of CO_2. Methane did not show a significant effect on ARA. The landfill gas levels in the landfill topsoil were > 10% O_2 and < 10% CO_2 in general; the nodules should fix N_2 over these ranges of gases.

The results of a four-week test showed that both CO_2 and landfill gas suppressed the growth of the two legumes and their nodular ARA. However, under the influence of the gases, infected seedlings had higher biomass than rhizobia-free seedlings.

The influence of landfill leachate on the same rhizobia-legume systems was assayed for five months with a serial dilution of leachate (73 - 0.58%). Although the ARA of the nodules was highly suppressed by the leachate, the harvestable biomass of inoculated seedlings was higher than rhizobia-free seedlings. The total N content in rhizobia-free seedlings was higher than inoculated seedlings, indicating that the legumes accumulated more N from leachate-contaminated-soil when they were not inoculated. Their performance was compared with two non-legumes (Cinnamomum burmanii, Tristania conferta). They tolerated the highest concentration of leachate for only one month and their total N content was less than rhizobia-free or infected legumes.

The growth of the two legumes under actual landfill conditions was investigated by transplanting rhizobia-free and pre-inoculated seedlings to two landfill sites. After six months, most of the rhizobia-free seedlings became infected: Acacia confusa 63, 70%, Leucaena leucocephala 17, 89%, respectively on Junk Bay and Shuen Wan Landfill. Their ARA was similar to that of pre-inoculated seedlings. However, the harvested biomass of pre-inoculated seedlings was generally higher than that of rhizobia-free seedlings. The results indicate that there were free rhizobia at these landfill sites to infect the legumes. They formed effective nodules under landfill conditions.

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ABBREVIATIONS

Ar	argon
ARA	acetylene reduction assay or acetylene reducing activity
atm	atmospheric pressure
cm	centimetre
CH_4	methane
C_2H_2	acetylene
C_2H_4	ethylene
CO_2	carbon dioxide
d	day
d. wt	dry weight
°C	degree Celsius
EC	electrical conductivity
f. wt	fresh weight
g	gramme
h	hour
ha	hectare
He	helium
l	litre
mmol	millimole
µg	microgramme
µl	microlitre
µmol	micromole
min	minute
mol	mole
N_2	(di-) nitrogen
NH_4-N	ammoniacal nitrogen
nm	nanometer
NO_2-N	nitrite nitrogen
NO_3-N	nitrate nitrogen
O_2	oxygen
Pa	Pascal
ppt	part per thousand
ppm	part per million
v.p.m.	part per million in volume
PAR	Photosynthetically Active Radiation
p.s.i.	pound per square inch
rpm	round-per-minute
RH	relative humidity
s	second
S	siemen
SD	standard deviation of the mean
t	metric ton (1000 kg)
$v\ v^{-1}$	volume by volume
$w\ v^{-1}$	weight by volume
YMA	yeast-mannitol agar
YMB	yeast-mannitol broth

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

1.1 Landfill and tree growth

1.11 What is landfill?

Landfill is a method of co-disposal of municipal waste and soil on land. It is the only way for ultimate disposal of waste in a controlled manner which causes the minimal nuisance to public health or safety. It has been called sanitary landfill or controlled tripping; however, it has become more common simply to call it "landfill" and it refers to the practice and those sites which do not accept hazardous or radioactive waste.

There are two extreme directions in landfill design: 1) attempt to isolate the waste being embedded inside from outside, as far as technology can achieve and in an economic way; 2) consider landfill as a bioreactor and try to maximize the degradation rate of waste. In Hong Kong, UK, USA and also many cities in other countries, the first idea commonly has been adopted. With such design, an impermeable bottom liner is laid at the bottom to avoid seepage of leachate into ground water or adjacent sea water (Seymour, 1992). The top of the landfill (landfill cap or final cover) is covered by gas-impermeable layers of soil and compacted by machinery (Department of the Environment (UK), 1994). Between the bottom liner and the landfill cap is a network of pipes to collect leachate and gas. The top liner not only avoids the migration of gas into the top soil, it also prevents the infiltration of rain water into the landfill. If rain water is allowed to contact the waste, the degradation rate of the waste will be increased and the amount of leachate to be collected for treatment will also be increased. However, due to the presence of the landfill cap, the moisture retained inside the landfill cannot percolate effectively to the topsoil, thus water supply for plants is generally inadequate, especially in the dry season.

The daily operation of a landfill site is to spread and compact the waste after loading from vehicles by waste-moving equipment into a layer of 1 m thick. The layer of waste will then be covered with soil about 0.2 m thick and further compacted by compactors. Hence, landfill cells of layers of waste and soil are formed. The final top cover is a layer of soil about 20 cm to 1 m in depth. To prevent differential settlement, a high degree of compaction over the whole area of the cell is maintained. An in situ density of 1.2 t m^{-3} can be achieved by a bulldozer (Department of the Environment (UK), 1994). Where densities of only 0.6 - 0.7 t m^{-3} were achieved, settlement of 20% or even greater is expected; on sites where the high density of 1.0 - 1.2 t m^{-3} is achieved, the settlement may be 10% or less (Street & Dumble, 1990). Others factors are considered in the planning, design and development of an landfill, including engineering aspects, mode of landscaping, environmental impact, operational issues and legal questions were discussed in Street and Dumble (1990) and Department of the Environment (UK) (1994).

1.12 What is the problem?

Although the design and practise of landfill in different countries are not the same, the basic problem of landfill gas and leachate contamination are in common (Lisk, 1991). Completed landfill sites are not suitable for large-scale construction due to the uneven settling of the site for more than 30 years and the presence of explosive landfill gas (Department of the Environment (UK), 1994). They are commonly converted into parks, golf courses, or botanical gardens. Such development generally involves woodland development and high mortality rate in tree planting has been reported in Hong Kong (Wong & Yu, 1988b) and in landfill sites in other countries (e.g. USA: Gilman et al., 1985; Leone et al., 1982; UK: Dobson & Moffat, 1993).

1.121 Landfill gas

The biodegradation of waste generates landfill gas. The typical composition of pure landfill gas is about 60% CO_2 and 30% CH_4 (v v^{-1}); however, concentrations as high as 89.3% CO_2 and 77.1% CH_4 have been detected (Department of the Environment (UK), 1994). The same report also indicated that in addition to the major components of gases present in the landfill cover soil, 108 organics were quantified and their levels were mainly < 0.1 mg m^{-3}. The production of the gases depends on climatic factors and the chemical nature of the waste. On landfill sites with advanced civil design, most of the gas is vented to the ambient or is collected for energy production. However, no matter how advanced is the civil engineering technology being applied on a site, a gas problem still exists in sites where the clay layer is not formed properly or is cracked by uneven settlement of the site. In the landfill cap, landfill gas is diluted by the ambient air. Therefore, the landfill gas concentrations collected by sub-surface probes are generally < 50% CO_2 and < 50% CH_4. Moreover, some of the CH_4 is converted to CO_2 by methanogenic bacteria (Fielding et al., 1988). This process decreases the concentration of CH_4 and increases the concentration of CO_2 in soil. Table 1.1 compares the major components of landfill gas reported in Hong Kong and in other cities.

Table 1.1 Typical landfill gas composition. All values as % v v^{-1}.

determinand	country and reference		
	Hong Kong	UK*	USA
	(Wong and Yu, 1988a; Sin, 1981)	(Dobson & Moffat, 1993)	(Lisk, 1991)
CO_2	12.6 - 28.2	63.8	47
CH_4	29.0 - 55.3	33.6	47.4
O_2	1.3 - 4.5	0.16	0.8
N_2		2.4	3.7
H_2		0.05	0.1
CO		0.001	0.1
ethane		0.005	
ethylene		0.018	
acetaldehyde		0.005	
propane		0.002	
butane		0.003	
He		0.00005	
higher alkanes		< 0.05	
unsaturated hydrocarbons		0.009	
halogenated compounds		0.00002	
hydrogen sulphide		0.00002	0.01
organosulphur compounds		0.00001	
alcohols		0.00001	
paraffin hydrocarbons			0.1
aromatic hydrocarbons			0.2
trace compounds			0.5
others		0.00005	

1.122 Landfill leachate

Rain water mixing with the soluble portions of waste and its by-products after degradation forms landfill leachate. Table 1.2 compares the chemical composition of leachate from different cities. The property of leachate depends highly on the nature of the waste disposed of in a site and also depends on the civil design of a site. High NH_4-N is a general characteristic for landfill leachate; it is not uncommon to have a leachate in excess of 1000 mg l^{-1} NH_4-N (Robinson & Gronow, 1992).

The problem of leachate seepage normally happens on confined areas and usually at the edge of a site. "Leachate breakout" is caused by high leachate levels within the waste and ingress into a site through weaknesses in the cap, or on an uncapped site (Dobson & Moffat, 1993). The breakout can cause injury to trees as a result from leachate toxicity. In West Virginia, USA, Menser et al. (1983) irrigated six tree species for 4 years with leachate and this resulted in significant mortality. In Hong Kong, a subtropical city, Wong and Leung (1989) irrigated leachate on Acacia confusa and this resulted in growth depression to about 25% of the control after 50 days. However, when the leachate concentration and irrigation rate were properly controlled, the presence of leachate could benefit the growth of plants. In Finland, higher coppice yields were reported after leachate irrigation (Ettala, 1988c). In Ontario, Canada, Cureton et al. (1991) found that irrigation of poplar and willow for two seasons stimulated height growth by 42% and 141%, respectively. Similar work by Gordon et al. (1989), also in Ontario, reported that red and sugar maple seedlings increased in stem diameter within 7 weeks.

Table 1.2 Composition of leachates from different countries. Units in mg l^{-1}, except for pH or where specified. # = ranges in which 70% of the literature values in USA.

Determinand	Hong Kong, (two sites, Robinson, 1991)	UK, Bassett Landfill (Robinson & Gronow, 1992)	USA "Typical concentration range" (Lu et al., 1984) #
pH	7.6-8.6	7.1	5-7.5
alkalinity	3230-11700	563	
EC (µS cm^{-1})	6340(?)-30400	13300	2000-8000
Cl	522-2740	1810	100-2000
total alkalinity			500-10000
total solids			3000-50000
total dissolved solids			1000-20000
suspended solids	3-124		
COD	641-2830	1280	1000-50000
TOC		1840	700-10000
BOD	57-384	102	1000-30000
total Kjeldahl-N	889-2860		10-5000
NH_4-N	784-2700	969	
NO_2-N	<0.1	0.06	
NO_3-N	<0.1-2.5	0.5	0.1-10
total-P	9.8-125	2.7	0.5-50
SO_4-S		26	10-1000
Na	217-2100	1320	200-1500
Mg		159	30-500
K	313-1130	858	
Ca	22.5-42.5	94	100-3000
Cr		0.06	0.05-1
Mn		0.28	
Fe	5.1-8.5	9.8	10-1000
Ni		0.12	0.1-1
Cu		0.17	0.02-1
Zn	0.2-2.2	0.62	0.5-30
As		0.021	
Cd		0.01	0.001-0.1
Hg		<0.1	
Pb		0.13	0.1-1

In addition to the effects on plant growth, research has been conducted on leachate with special emphasis on its recirculation on landfill sites as an alternative approach to leachate treatment (Cureton, 1991). It was found that leachate recycling can increase the rate of biodegradation of organics and the landfill would become "stabilized". When leachate recycling was practiced, CH_4 production could be shortened from 20 to 30 years to a few years (Lee et al., 1986). Leachate recycling has also been practiced as a method of leachate disposal; the application of leachate to the surface of a landfill can result in significant decreases in leachate volume due to evapotranspiration. However, municipal sanitary landfill represent a threat to groundwater quality; therefore, to prevent further groundwater contamination, leachate recycling is not commonly adapted in cities demanding groundwater for their main water supply. For example, leachate recycling have been banned in New Jersey (Lee et al., 1986). However, Hong Kong depends on neither groundwater for domestic nor industrial use.

1.123 Other landfill factors

In addition to gas and leachate, the landfill environment also has quite a lot of special characteristics which affect the growth of trees. Waterlogging is common in temperate landfill, where the evaporation rates are low (Dobson & Moffat, 1993). The problem of drought was reported in subtropical landfill by Wong and Yu (1988b). Another common feature is the high bulk density in cover soil caused by compaction of soil and waste. Excessive compaction of soil might hinder the penetration of common plant roots. Zisa et al. (1980) demonstrated that the growth of Austrian pine roots on a silt loam and sandy loam restricted at bulk density of 1.4 to 1.8 g cm^{-3}. Heiman (1981) reported that the penetration of Douglas fir seedlings started to decline when the bulk density was higher than 1.37 g cm^{-3}. The restriction of root growth not only depends on bulk density, but also on soil particle size. Plant roots will rarely penetrate into light textured soils having a bulk density of greater than 1.7 - 1.8 g cm^{-3} or heavy textured soils with a bulk density greater than 1.5 - 1.6. Elevated temperature, caused by the heat-release process of the biodegradation of waste, is another common feature on landfill sites. The influence of elevated temperature was summarized by Dobson and Moffat (1993).

1.13 The problem in Hong Kong

1.131 General review

Hong Kong is one of the earliest cities in the Far-East to employ landfill technology in waste disposal. Thirteen landfill sites have been operated since 1973. In 1992, Hong Kong generated daily 8000 t municipal solid waste (EPD, 1993a) and this value is expected to increase annually by 3.5% in the coming 15 years. Except for the 13% of the waste which was incinerated, the waste has to be disposed into landfill sites. Furthermore, the two incinerators operating in early 1994 will soon be phased out to minimize air pollution in the city, when the long-term waste management facilities become operational. The long-term strategy of solid waste treatment is to dispose of all municipal waste into three strategic landfills: South East New Territories (SENT) Landfill, West New Territories (WENT) Landfill, North East New Territories (NENT) Landfill (EPD, 1992, 1993a). The WENT Landfill started its operation in November 1993 and was estimated to have a capacity of 57,000,000 m^3 and life of 25 to 30 years (Anon., 1992b). However, before the full operation of these three strategic landfills, the main portion of waste still has to be disposed into three landfill sites operated for seven to ten years: Junk Bay, Shuen Wan, Pillar Point.

1.132 The 13 landfill sites

The 13 landfill sites mentioned in the preceding section cover a total area of 268.1 ha (about 2.0% urban area of the city (Webb, 1991)). They can be classified into four categories: valley fill (e.g. Siu Lang Shui), quarry fill (e.g. Ma Yau Tong Central), build-up/platform (e.g. Gin Drinkers' Bay), marine fill (e.g. Shuen Wan) (Table 1.3). The special features of each site are described in ascending order of their closing dates (EPD, 1987; EPD 1993 a & b):

Ngau Tam Mei

This small landfill was the first completed site in Hong Kong. The operation started in 1973 and was closed in 1975 shortly after 1.5 years. It had been used as an open-storage site by the Hong Kong Government for an uncertain period after its closure.

Ngau Chi Wan

It is the only completed site converted to a public facility for coach parking; all other sites are restricted areas and unlicensed access is prohibited. It was closed in 1977 and is the first completed urban site.

Gin Drinkers' Bay

This is the first large-scale completed site, and many tree species have been planted there in order to search for the best species for growth on similar completed sites. Within the first 15 years, at least 13000 tree seedlings of about 30 species were planted on this site; of which eight were legumes. The original plan for the site development was to convert the site into a park with playground and golf courses by 1991. The civil and tree planting work had been completed by 1992. However, due to the problem of landfill gas migration, the park still could not be opened for the public by 1994 (Pugh & Choi, 1993).

Ma Tso Lung

This is a remote site located adjacent to the northern border in the New Territories. This is also one of the smallest sites (2.0 ha). Starting from 1976, it received waste for 2.5 years and was closed in early 1979.

Sai Tso Wan

A network of active gas abstraction system was installed in this site and landfill gas was directed to a gas abstraction plant. This is the only site with such gas control and abstraction design.

Ma Yau Tong West

It was proposed that the completed site should be converted into the Junk Bay Road Park. Before it was converted into a landfill site, the northern part of the site was used to be a small reservoir.

Siu Lang Shui

Most of the domestic waste collected from the North-West of Hong Kong was disposed in this valley-fill site before summer 1983.

Ma Yau Tong Central

The landfill operation in this site was commenced in 1981 upon the closure of the nearby Ma Yau Tong West site. The operation ceased after five years and was used as a temporary tyre storage area for years.

Jordan Valley

The exact month of closure of this site is not available, but is very likely being closed shortly before 1988. This site is located in the northern border of the Kowloon City and is becoming enclosed by residential buildings.

Shuen Wan

By 1994, the Shuen Wan Landfill has been operated for twenty years. Before the landfill operation, open dumping was practiced for years. It was one of the four marine/reclaim sties. The final dam which fixed the site area was completed in 1993.

Junk Bay Stage I

This site was operated for about 14 years. Before the chemical waste incineration plant in Tsing Yi Island started its operation in late 1993, the site received for years most of the chemicals and hazardous wastes generated in Hong Kong. However, in terms of quantity, domestic and construction wastes were far more than the special wastes being disposed on this sites.

Junk Bay Stage II/III

The exact month of opening of this site is not available. However, its operation was after the Junk Bay Stage I. The site area is about one-half of the Junk Bay Stage I and they are about 400 m apart.

Pillar Point Valley

This is one of the four on-going sites and receiving waste generated from the New Territories. It is also the only inland (non- marine/reclaim) on-going site. It started operation in 1983, a few months before the closure of the Siu Lang Shui Landfill.

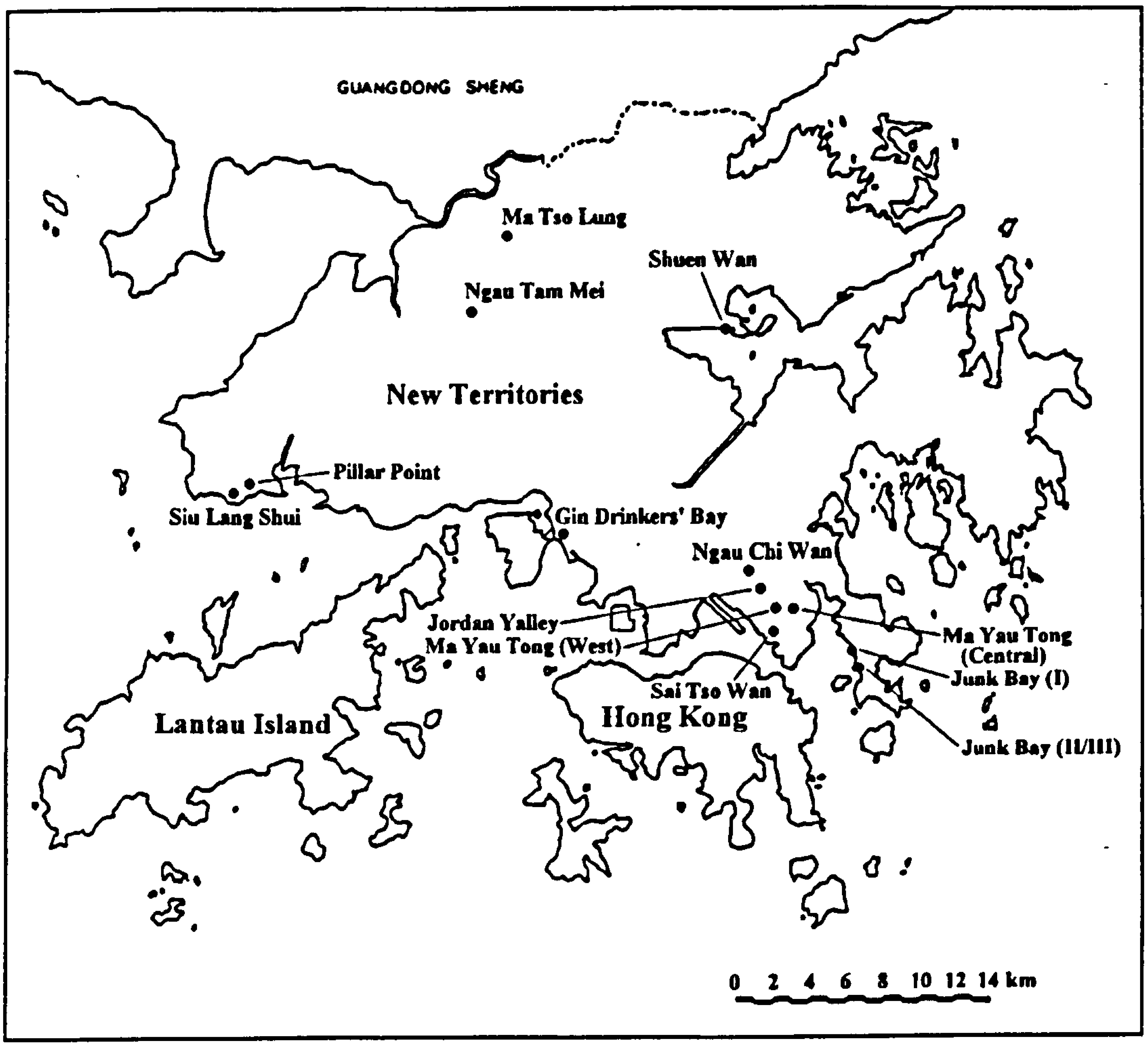


Fig. 1.1 Locations of the 13 landfill sites in Hong Kong.

Table 1.3 Site history and details of the 13 landfill sites. * Open dump initially until converted to controlled tip in the stated months, – = no information

	Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers' Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
site area (ha)	2.0	13.5	29.0	2.0	14.0	6.6	11.7	5.8	6.5	48.0	60.0	35.0	34.0
landfilled quantity of waste (Mt)	0.03	0.734	3.54	0.182	1.59	0.595	1.19	1.05	–	–	–	–	–
opening date	12/73*	1/76	6/73*	7/76	3/77	2/79	11/78	2/81	4/86	6/74*	3/80	–	8/83
closing date	3/75	12/77	2/79	2/79	2/80	2/81	12/83	4/86	before 1988	on-going	on-going	on-going	on-going
predevelopment terrain	shruband	shrubland	marine and reclaim	agricul-ture	terrace	terrace	shrubland	quarry	–	marine and reclaim	marine and reclaim	marine and reclaim	–
type of site	valley fill	valley fill	built-up platform/ marine fill	valley/ built-up platform	valley/ built-up platform	valley fill	valley fill	quarry fill	quarry fill	marine fill	marine fill	marine fill	valley fill

1.133 In search of suitable species

Nine of the 13 landfill sites in Hong Kong have been completed more than eight years. The Hong Kong Government planned to convert most of the sites into parks and open for public (EPD, 1993b). However, many difficulties were encountered in getting the suitable species for the landscaping work. The adverse tree growth on Hong Kong's completed landfill sites have been described by Chan et al., 1991; Wong & Leung, 1989a; Wong & Yu, 1988b. Moreover, Chan (1989) compared ten species of tree and reported that legumes were more suited than non-legumes in general, but the N_2-fixation of the legumes was not tested.

As four species of tree were used intensively in the research work to be described in Chapters 4 to 6, it is necessary to provide some basic information in this Chapter:

Acacia confusa

This legume belongs to the Mimosaceae. The alternate plyllode leaf is the photosynthetic organ. It is simple, 6 to 11 x 1 to 1.5 cm in size, without a blade and crescent shaped. It is a graceful, evergreen, bushy tree, native of Taiwan and the Philippines. A mature tree can easily attain a height of 6 to 15 m, bearing many slender, somewhat crooked branches. It grows well in Hong Kong in almost any position and is widely planted in groups. As it is an excellent wind-break, but not a good shade tree, it is more suited for planting on hillsides than in parks and gardens (Thrower, 1977, 1988; Urban Services Department, 1969). Its high tolerance to landfill conditions was reported by Chan et al. (1991).

Leucaena leucocephala

A small Mimosaceae evergreen legume originating from tropical America and now distributed throughout the tropics. It is the type species of the genus described by Lewis and Elias (1981). A mature tree can reach 9 m high, but does not provide thick cover. Although it has become naturalised in Hong Kong, being a common roadside plant and often forming a dense woodland in waste lowlands or hillside, it was neither supply tree list by the Government Nursery for landscaping purposes nor could be purchased from any private sector in Hong Kong (Thrower, 1977, 1988).

Cinnamomum burmanii

The common name of this tree is cinnamo tree and it belongs to the Lauraceae. It is a native evergreen tree. A mature slender tree may reach 5 m high and has purplish-brown branches. The leaves are opposite, simple, up to 10 cm long and half as wide. It flowers in March in large terminal or axillary clusters, usually shorter than the leaves. The fruit is a black berry, small and oval in shape. As it is a native, it is used commonly in Hong Kong mixed with other trees for woodland establishment (Thrower, 1977, 1988).

Tristania conferta

This originates from Australia and may attain a height of 40 m in its native habitat. In Hong Kong, it is evergreen and fast-growing, reaching 6 m. The adult leaves are alternate, grouped in whorls at the end of each season's growth, elliptical, 7 - 15 x 2.5 - 6 cm. It flowers in early summer and the fruit is a woody capsule 1 - 2 cm long, oval or bell-shaped. It is widely used in afforestation in Hong Kong and often mixed with pines (Pinus spp.). It suits a hot and dry climate, grows in any soil, but prefers a sheltered position with good soil. Although it does not spread enough to be a shade tree, it yields an excellent timber valued for its strength and durability (Thrower, 1977, 1988). It has been tested and is suited for growth on completed landfill sites with a climate similar to Hong Kong (Chan et al., 1991).

1.14 The problem in other countries

Landfill development is increasing both in number and in size in most cities of the world. As a direct consequence, more completed landfill sites are expected to need redevelopment.

In 1985, most of the domestic waste collected in the cities of China was disposed by open dumping and only 1.6% was treated in a sanitary way. In Guangdong, the province of China next to Hong Kong, the first landfill site was commenced in 1985 (Chau, 1985; Fung, 1985) and landfill has started to become the chosen waste disposal method in most other cities in China in the past few years. Bangkok has started to employ landfill for final waste disposal since 1990 and treated about 5% of the city daily output in the same year (Anon., 1991a). In 1990, Singapore generated 5500 t d^{-1} of waste and 42.8% was disposed of at the landfills; the land-limited Singapore plans to develop new landfill on offshore islands (Anon., 1991b). Taiwan generated 7×10^6 t of solid waste in 1991, 30% up on 1987 figures (Anon., 1991c). According to the National Six-year Plan (1992 - 1997) in Taiwan, 55 major landfills and 23 regional landfills are to be built to deal with about 50% of all Taiwan's waste; the rest is mainly to be incinerated (1992a). In Japan, the refuse is mainly handled by incinerators. Korea produced 90000 t waste a day in 1991, one-third being collected in Seoul and disposed mainly in the capital's Nanjido landfill. This landfill was overflowing and to be replace by the Kimpo (Anon., 1992a).

In UK, landfill remains the principal means of waste disposal (approx. 90%) (Street & Dumble, 1990). The largest site currently run in UK is in Packington, it has an area of 155 ha, 50 m high, accepting 1.5×10^6 t $year^{-1}$ and generates 3.7 Mwatt of power from landfill gas (Dobson & Moffat, 1993; Street & Dumble, 1990). To protect the environment, an assessment of the impact of the scheme on the ecology, both within the site boundaries and in the vicinity of the site are required in UK (Street & Dumble, 1990). Difficulties in woodland establishment on completed landfill sites in the UK were described by Dobson and Moffat (1993). This also listed suitable and unsuitable trees for planting. According to the authors, five of the 30 "likely to be tolerant" trees are legumes.

The difficulties in tree planting on USA landfill sites (mainly in New Jersey) are given in Flower et al, 1981; Gilman et al, 1982; Lisk, 1991. Etalla (1987, 1988c) and Etalla et al. (1988a, b) described the tree growth on Finland landfill sites. The effects of leachate recirculation on willow were reported by the same authors. The effects of leachate recirculation on temperate species were also reported by Gordon et al. (1989) and Cureton et al. (1991).

1.2 Biological nitrogen fixation

The process by which dinitrogen is reduced biologically to NH_4^+ is called dinitrogen fixation or simply nitrogen fixation (N_2 fixation). This process requires an enzyme complex called nitrogenase, which consists of two proteins: Fe protein; Mo-Fe protein (Shirlov, 1978). The Fe protein contains four Fe atoms per molecule and also sulphide and thiol groups. The Mo-Fe protein contains two Mo and 24-36 Fe atoms per molecule and sulphide and thiol groups. Neither the Fe protein nor the Mo-Fe protein individual fraction has biological activity; when separated proteins are restored, ARA is also recovered (Hardy et al., 1968).

The overall chemical reaction for nitrogen fixation (reduction) is summarized (Salisbury & Ross, 1992) as:

$$N_2 + 8\ e^- + 16\ MgATP + 16\ H_2O \xrightarrow[\text{nitrogenase}]{} 2\ NH_3 + H_2 + 16\ MgATP + 16\ P_i + 8\ H^+$$

In addition to reducing N_2 to NH_4^+, nitrogenase can catalyze the reduction of acetylene (C_2H_2) into ethylene (C_2H_4) (Balandreau, 1980; Bergersen, 1970). This process is commonly used to quantify N_2 fixation of biological samples. The concentrations of C_2H_2 and C_2H_4 can be determined accurately by a gas chromatograph.

$$C_2H_2 + 2\ H^+ + 2\ e^- \xrightarrow[\text{nitrogenase}]{} C_2H_4$$

1.21 Rhizobia-legume symbiosis

In the past, the sole criterion for the genus Rhizobium is the ability of the bacterium to form nodules on members of the Fabaceae (Trinick, 1982). However, the attempt to group all bacteria that infect members of Fabaceae into one genus has caused confusion and much dissatisfaction with the taxonomy of Rhizobium. In the Bergey's Manual of Determination Bacteriology, 8th edn (Jordan & Allen, 1974), two genera were included in "the Rhizobium family" Rhizobiaceae: Rhizobium, Agrobacterium. However, Agrobacterium spp. do not stimulate root nodule production on leguminous plants. The same manual listed six species under Rhizobium: R. leguminosarum; R. phaseoli; R. trifolii; R. meliloti; R. japonicum; R. lupini. This system was commonly followed and each species are designated to its host range (Allen & Allen, 1981; Dazzo, 1982; Lopes, 1977). Recent classification does not group all Rhizobium-like species into one genus, e.g. Bradyrhizobium and Azorhizobium also can form nodules on Fabaceae (Trinick, 1982). Therefore, Jordan (1984) revised the family Rhizobiaceae in the latest edition of Bergey's Manual. Four genera are included in this family: Rhizobium, fast growth and cause nodule production on roots of leguminous plants; Bradyrhizobium, slow growth and cause nodule production on roots of tropical and some temperate zone leguminous plants; Agrobacterium, cells cause root galls but within N_2-fixing activity; Phyllobacterium, cells do cause leaf nodules. The six species of Rhizobium in the 8th edn of Bergey's Manual were retained (Jordan, 1984). The identification process of Rhizobium or Rhizobium-like species is quite complicated and must involve reinoculation on its host. Nodule-forming bacteria isolated from the site are usually simply called a Rhizobium species or Rhizobium-like species; the host must be named but the species is sometime not classified. A particular Rhizobium or Rhizobium-like species is generally effective with only one legume species. The rhizobial infection process was described by Kijne (1992) and cross-inoculation among rhizobia and legumes was described by Bülow (1977).

Rhizobia growing on yeast-mannitol agar (YMA) are small to medium-sized gram-negative rods, varying in width from 0.5 - 0.9 µm (Weaver & Frederick, 1982). They occur singly or in pairs and can often appear in groups with a side-by-side arrangement. The size of the rhizobial cells varies according to the physiological age of the culture. Young cells are frequently motile and have peritrichous, polar, or subpolar flagella

(Jordan, 1984; Trinick, 1982). A detailed description of the infection process of rhizobia on legumes can be found on Kijne (1992) and the confirmation of nitrogen fixation in bacteroid cells was summarized by Evans and Burris (1992).

Polhill and Raven (1981) grouped all leguminous plants into the family Leguminosae with three subfamilies: Caesalpinioideae, Mimosoideae, Papilionoideae. However, Papilionaceae - Papilionoideae are traditional names for family - subfamily accorded to the papilionates and the alternative names Fabaceae - Faboideae are also widely used (Polhill & Raven, 1981; Polhill, 1981). Nodulation is general in Mimosoideae and Papilionoideae, but less common in Caesalpinioideae (Polhill et al., 1981; Trinick 1982). Detailed nodulation data of different legumes are available in Allen and Allen (1981). There are three principal geographic regions where caesalpinoid legume genera abound – South America, tropical Africa and Southeast Asia (Cowan, 1981). Cassia and Bauhinia are two of the larger genera in the subfamily Caesalpinioideae occurring throughout the tropics. The Mimosoideae contain approximately 50 - 60 genera distributed throughout tropical, subtropical and warm-temperate regions of the world. The three major genera of this subfamily constitute almost two-thirds of the known legume species: Acacia with 1200; Mimosa with 400 - 500, and Inga with 350 - 400 (Elias, 1981). The subfamily Papilionideae comprises some 440 genera and 1200 species, widely distributed from rain-forest to the edges of dry and cold deserts (Polhill, 1981). Rain forest trees and lianes occur principally in Amazonia and around the Gulf of Guinea.

1.3 Factors affecting rhizobia-legume symbiotic nitrogen fixation

1.31 Gaseous factors

1.311 Oxygen

The atmospheric ambient concentration of O_2 is 20.95% (v v^{-1}).

Nitrogen fixation is sensitive to O_2 because the Fe protein and Mo-Fe protein of nitrogenase can be denatured oxidatively by O_2 (Salisbury & Ross, 1992). The purified Fe proteins can be rapidly inactivated by O_2 and are unstable to prolonged storage except in bead form and in liquid nitrogen. Eady and Smith (1978) reported that 50% of the activity of highly purified Mo-Fe proteins was irreversibly inactivated by O_2 at 30°C within few minutes. Bergersen (1970) also reported that a bacteroid suspension started to decline in reducing activity above 8% O_2. However, O_2 is essential for bacteroid respiration. For aerobic N_2-fixing system, a fall in O_2 concentrations due to respiration will cause a decline in the rate of ARA (Turner & Gibson, 1980).

In order to prevent the nitrogenase being oxidized by the O_2, legume root nodules develop several mechanisms to protect the nitrogenase. The nodule cortex is a barrier for O_2 diffusion; a nodule can adapt to increased external O_2 by regulating its resistance to O_2 diffusion (King et al., 1988). Koch and Evans (1966) reported that excised soybean nodules fixed N_2 for 2 h. However, the rate of fixation was greatly reduced in sliced nodules and fixation was virtually lost when nodules were crushed. Inside the nodule cortex cells occurs the protein leghaemoglobin, which gives legume nodules a pink colour (Evans & Burris, 1992). The function of leghaemoglobin is to control the transfer of O_2 to the nodule interior (Becanna & Klucas, 1992; Werner, 1992). Respiration is another protective mechanism to maintain the O_2 in a nodule at a low level (Castillo et al., 1992).

Different legume nodules have different optimum O_2 concentrations for N_2 fixation. However, different laboratories have also reported different optimal O_2 concentrations for the same species. For example, Bergersen et al. (1973) reported 100% O_2 was optimal for the ARA of soybean. The optimal O_2 concentration for the same legume reported by Masterson and Murphy (1980) was 50%. Lower concentrations of 30 and 20% were reported by Heckmann and Drevon (1988) and King and Layzell (1991) and 5 to 30% was reported by Criswell et al. (1976). However, Hardy et al. (1973) summarized that common legume nodules require aerobic conditions for ARA and a O_2 concentrations of 20 to 50% are optimal. The relationships between high external O_2 tension and nodule/nitrogenase activities have been studied intensively by Bergersen, 1962, 1970; Bergersen et al., 1973; Hardy, 1968; Masterson & Murphy, 1976 and Turner and Gibson 1980. The minimum O_2 concentration for ARA was recommended to be 18.8% (Hunt & layzell, 1993).

When the O_2 level is less than optimal, N_2 fixation declines. Bergersen et al. (1973) reported a drop of 42% of the ARA of soybean nodule when the O_2 concentration dropped from 28% to 9%. Generally, under a subambient partial pressure of O_2, the N_2 fixation by a nodule is proportional to the O_2 concentration (Hardy et al., 1973; Heckmann & Drevon, 1988; Masterson & Murphy, 1980; Waughman, 1972).

Many factors can affect the response of nodule to external O_2 levels. Older nodules showed oxygenation at a lower O_2 concentration (Bergersen, 1962). The O_2 stress seems can be adapted by nodules, Dakora et al. (1991) reported that cowpea and soybean nodules adapted to extreme O_2 concentrations within the range of 1 to 80% O_2 and neither the proportional composition of leghaemoglobin nor their oxidation state was affected after 28 d.

1.312 Carbon dioxide

The atmospheric ambient concentration of CO_2 is 0.035% (v v^{-1}).

The influence of CO_2 on the growth and activity of plant and nodules is quite complicated. In plant cells and in bacteroids, CO_2 can exert its influence in the form of gaseous CO_2 or bicarbonate. It is not easy to identify the influence of CO_2 on a cell is due to the direct effect of the gas or in the form of HCO_3^- ion which affects the pH of the cell sap. Moreover, CO_2 has both stimulatory and inhibitory effects, depending on the levels of the gas and the duration of exposure. The different responses to CO_2 on different plants make the study of this gas more difficult. For example, Pearce and Jackson (1991) reported that CO_2 promoted rice coleoptile extension but has less effect on barnyard grass (Echinochloa oryzoides).

Low levels of gaseous CO_2 or bicarbonate can stimulate the cellular respiration of a plant cell. It was reported that 0.1% CO_2 increased the yield of Rhizobium cells (Heckmann & Drevon, 1988) and CO_2 in the root environment of pot-grown legumes promoted N_2 fixation (Turner & Gibson, 1980). Ortega et al. (1992) also reported that an increase in CO_2 concentration from ambient to 0.1% not only promoted early bacteroid development and nitrogenase induction, but also provided more fixed N for incorporation into bean (Plaseolus vulgaris L.) fresh weight. Concerning the biochemical pathways of a bacteroid, Heckmann and Drevon (1988) reported that the addition of 0.46% CO_2 stimulates phosphoenolpyruvate (PEP) carboxylase activity and increased the flow of malate, a major source of energy for bacteroids, and consequently increases nitrogenase activity. In this process, CO_2 is fixed by the microorganisms to replace the TCA cycle intermediates (Gaudy & Gaudy, 1981). PEP can be converted from a number of compounds, e.g. oxaloacetate, pyruvate and lactate (Lehninger, 1975). In addition to malate, the bacteroid can make use of other carbohydrates (Evans & Burris, 1992; Heckmann & Drevon, 1988).

An inhibitory effect of CO_2 (bicarbonate in the cell) on the cytochrome pathway of plant cells has been reported. Palet et al., 1991 indicated that the cytochrome pathway can be inhibited partially by CO_2 (mainly free CO_2). It has been suspected that the CO_2 concentration directly affects respiration and also affects the rate of an energy-requiring process, whose ATP or NaDPH demand affects respiration. Reduction by CO_2 ($\cong$ 0.12%) in dark respiration was demonstrated in alfalfa seedlings and the effect was more pronounced with seedlings grown with rhizobia than NO_3^- (Reuveni & Gale, 1985). Other inhibitory effects of CO_2 (bicarbonate in cell) on the cytochrome pathway (Palet et al., 1991), malic enzyme (Kerbel et al., 1988) and succinate dehydrogenase (Shipway & Bramlage, 1973) have also been reported. However, the mechanisms by which CO_2, especially at a high concentration, differentially affects tissue respiration are still not well understood.

1.313 Dinitrogen

When C_2H_2 and N_2 are both present, they compete for electrons in the nitrogenase reaction. It was reported that failure to replace air with an inert gas-O_2 mixture before ARA reduces $N_2(C_2H_2)$-fixing activities of soybean nodules by 10 to 20% (Hardy et al., 1968).

1.314 Acetylene

Acetylene (C_2H_2) is about 60 times as soluble in water at 25°C as is N_2. Acetylene pressure at 10 to 20% in ARA should produce saturation comparable to 80% N_2 for in vivo and in vitro nitrogenase. In soybean nodules, the ARA was not affected at 10% C_2H_2 even when the O_2 level was as high as 90%, but the reduction ability started to decline at 40% O_2 when the C_2H_2 level was 10% (Bergersen, 1970) This was due to C_2H_2 being about 33 times more soluble than O_2 (Bergersen et al., 1973). Maximum concentration of 5% C_2H_2 was recommended for common ARA study (Turner & Gibson, 1980).

1.315 Ethylene

Ethylene (C_2H_4) is a plant hormone and an inhibitor for nodulation. The nodulation to a low-nodulation pea mutant was restored by inhibitors (Ag^+, Co^{++}) of C_2H_4 formation or action (Fearn & LaRue, 1991). Nitrate inhibition of nodulation is mediated by C_2H_4 (Nigero et al., 1991).

The presence of C_2H_4 as a product of ARA, especially on prolonged ARA, may affect the intact plant activities, e.g. causing epinasty on leaf. Therefore, prolonged ARA and using intact plant is not encouraged.

1.32 Physical factors

1.321 Temperature

The maximum nitrogenase activity of common Rhizobium cells lies between 20° to 30°C (Hardy et al., 1973; Masterson & Murphy, 1980; Jordan, 1984). The effect of temperature on nitrogenase activity was described by Waughman (1977). Some rhizobia can tolerate higher temperatures. For example, Jordan (1984) reported that R. meliloti tolerated up to 42.5°C and R. leguminosarum 38°C. High soil temperature of 42°C at 4 cm depth, which is common in subtropical soil, was reported to reduce the inoculation rate of rhizobia on Glycine max (Ham, 1980).

1.322 Light

The principal relationship between light and ARA concerns its role in the supply of energy and reductant in photosynthetically active organisms (Masterson & Murphy, 1980; Ryle, 1988). Legumes and non-legumes placed in the dark lose $N_2(C_2H_2)$-fixing activity rapidly within 12-24 h (Hardy et al., 1973). The loss was correlated with the low sucrose levels. Such rapid responses to light demonstrate the need for adequate control of samples spaced throughout the day for assays and the need for prompt assay of nodulated legumes after removal from their native environment.

1.33 Other factors

1.331 Water

Water stress, whether due to deficiency or excess, has a profound effect on nitrogenase activity (Masterson & Murphy, 1980). Excess water affects ARA of the nodule through blocking the intercellular spaces and thus impeding diffusion of O_2 to, or C_2H_4 from, the bacteroids. This effect appears to be more marked with detached nodules than with nodulated roots. Water deficiency can cause osmotic imbalance, impair O_2 diffusion in the nodule and reduce the transport of fixation products from the nodule to the rest of the plant.

1.332 Combined nitrogen

Legumes in symbiosis with bacteria have two sources of N nutrition: fixation of atmospheric molecular N_2 and assimilation of soil N, found mostly in the form of NO_3^- (Heckmann & Drevon, 1988). However, combined N in soil inhibits rhizobia nodulation and N_2-fixation. Sheehy and McNeill (1988) demonstrated the addition of 220 mg NO_3-N l^{-1} effectively reduced acetylene reduction to zero and almost completely suppressed nodule formation on sainfoin plants. Inhibition of nodulation by NO_3^- is mediated through the phytohormone C_2H_4 (Ligero & Olivares, 1986; Ligero et al., 1987, 1991). It was proposed that internal root levels of isoflavonoids may be important in nodule development and Cho and Harper (1991) reported NO_3^- application markedly decreased isoflavonoid concentrations in non-inoculated soybean roots. When roots were inoculated, the nodule number, weight and nitrogenase activity were markedly suppressed by 5 mmol NO_3^-.

The inhibitory effect of NH_4^+ is less than that of NO_3^- at similar molar concentrations (Heckmann & Drevon, 1988). 50% inhibition in ARA was produced by 50 mM NH_4Cl on soybean nodules (Hardy et al., 1968). Ammonium is the normal product of N_2 fixation, but is not formed during C_2H_2 reduction, so repression-derepression effects of NH_4^+ on nitrogenase and possible effects of biosynthetic reactions of NH_4^+ on nitrogenase, are not encountered. This interruption in NH_4^+ formation increases in importance with length of incubation time and is a major reason for using only short-term incubation.

Other forms of combined N may also inhibit nodulation and N_2 fixation. Treatment of nodulated soybean plants with urea led to decrease in C_2H_2 reduction and in leghaemoglobin (Dalton et al., 1991).

1.333 Mineral

The rhizobia-legume system is less susceptible to fluctuation in mineral supply compared with free-living N_2-fixing bacteria due to the relatively stable supply of mineral from the host. Munns and Mosse (1980) indicated that legumes commonly demand higher levels of boron and P. Further detailed information of mineral requirement was described by the same report and compared on different symbiotic systems.

1.334 pH

The tolerance of pH extremes of rhizobia in media varies significantly between strains, generally rhizobia can grow at pH less than 5.5 and some can tolerate pH < 4.5 (Munns & Mosse, 1980). In the symbiotic system, rhizobia are protected by the host, but soil acidity usually reflects low levels of P, Mg and Ca concentrations and high levels of Mn and Al which is inferior to the growth of the host (Mannetje et al., 1980).

1.335 Injury

Excised nodules are less active than nodulated roots and whole plants may be even more active than nodulated roots. However, the use of nodulated roots are recommended for common ARA (Hardy et al., 1973).

1.335 Metals

Molybdenum, a component of nitrogenase, is the most specific metal for N_2 fixation (Burris et al., 1977). Ni has been demonstrated to be an important element in legumes and non-legumes; it plays a role in the metabolism of arginine released during protein degradation and turnover (Evans & Burris, 1992). Symbiotic assimilation of N_2 commonly creates higher demands of Cu and Zn (Munns & Mosse, 1980).

Growth of legumes and their related rhizobia is suppressed by different extents by different heavy metals. McGrath et al. (1988) reported that the growth of white clover was depressed particularly by Zn, to a lesser extent by Cu but not at all by Ni or Cr. Nodules from sewage sludge-treated soil were small, white, very numerous and evenly spread throughout the entire root system. This pattern is a characteristic of nodules unable to fix N_2 and repeated infection occurs because there is no feedback from effective nodules. The toxicity of metals in soil to plants is commonly collectively indicated by the value of Zn equivalent (= (Zn) + 2(Cu) + 8 (Ni)) (McGrath et al., 1988); a similar index of toxicity to the growth and activity of rhizobia is not available. However, reports on the comparative toxicity of metals to symbiotic N_2-fixation indicate that the overall order of decreasing toxicity is Cd > Ni > Cu > Zn > Pb (Vesper & Weidensaul, 1978; Sheridan, 1979; Porter & Sheridan, 1981; Päivöke, 1983a, b). Cr and Pb in particular are very insoluble in soil solution and neither is likely to have toxic effects on plants. The symbiotic N_2-fixing system is less sensitive to metal contamination than free-living N_2-fixing organisms as the bacteroids in the former system will be protected to some extent from soil metals by the host tissue whereas the later experience the direct influence of soil metals (McGrath et al., 1988).

will be protected to some extent from soil metals by the host tissue whereas the later experience the direct influence of soil metals (McGrath et al., 1988).

1.4 Aims

The establishment of a woodland is a feasible way to restore completed landfill sites and leguminous trees are generally suited for this (1.12, 1.133). However, there is apparently no information in the literature on N_2 fixation by leguminous plants under landfill conditions (1.133). The aims of this study were to investigate the role and importance of rhizobia-legume symbiotic N_2 fixation under landfill conditions. Both laboratory and field experiments were used to fulfill the aims and the organization of the experiments were:

1) to identify main limiting factors which affect tree cover and to compare the relative abundance between leguminous and non-leguminous trees on completed landfill sites;
2) to assess the effect of three major components of landfill gas (O_2, CO_2 and CH_4) on the growth of leguminous trees and on the N_2 fixation in their associated root nodules;
3) to compare the effect of landfill leachate on the growth of legumes and non-legumes and to investigate the influence of landfill leachate on nodular N_2 fixation;
4) to study the presence of free rhizobia in landfill soil and their ability to form effective nodule on legume hosts to fix N_2.

CHAPTER 2
MATERIALS AND METHODS

2.1 General procedures

2.11 Mass and volume determination

Chemicals, biological samples and laboratory wares with a mass less than 300 g were weighed to an accuracy of ± 0.1 mg on a AND model ER-180A top-pan analytical balance. Items exceeding 300 g were weighted on a Precisa 500M-2000C top-loading balance to an accuracy of ± 0.001 g. Objects weight refers to the amount of mass present, measured under the force of gravity.

For liquids less than 10 ml in volume, they were transferred by either class A glass pipettes or by a multi-dispenser. Glass measuring cylinders or class A volumetric flasks were used to measure samples exceeding 10 ml.

2.12 Biomass

Plant biomass refers to the 85°C oven dry weight (d. wt) of sample, dried on preweighed aluminium foil or a porcelain crucible for at least 48 h. Fresh weight (f. wt) was reported as the biomass of root nodules.

2.13 pH

An Extech model pH246072 pH meter with Cole Parmer G-05992-20 probe was used to determine the pH values of liquid samples. (Other instruments were occasionally used when the Extech machine was not available.) All instruments were calibrated with standard pH buffers before measurement. The pH values reported were the arithmetic mean via the antilogarithms of the readings of replicated samples (Grimshaw, 1989).

2.14 Quantum flux density

Quantum flux density was measured using the built-in quantum flux density meter in a Li-Cor steady state porometer and expressed as $\mu mol\ m^{-2}\ s^{-1}$ PAR.

2.15 Temperature

The temperature of the laboratory or a solution was measured by glass Hg thermometer. When continuous measuring was needed, it was monitored by an Isuzu thermohygrograph model 3-1125. All data reported are in degrees Celsius (°C).

2.16 Flame atomic absorption spectrophotometer

The concentrations of Ni, Cu, Zn and Cd in acid digested samples were analyzed with a Varian SpectrAA-20 atomic absorption spectrophotometer, operated with an air-acetylene flame. The machine was calibrated with a series of standard solutions prepared from 1000 mg l^{-1} metal standard solutions from Merck and recalibrated every 50 to 100 determinations of sample .

2.17 Culture vessels and laboratory ware

Both borosilicate and plastic glassware were employed. For quantitative analyses, laboratory glassware was prewashed with phosphate-free detergent and rinsed by distilled water. If necessary, laboratory wares were soaked in 4% HCl for at least 24 h to remove all trace of bound metals, P and minerals. Acid treated utensils were then rinsed with distilled water and dried at 60 - 90°C until dry. Apparatus composed of rubber was not acid washed.

2.18 Sterilization

Procedures which required sterile conditions were carried out either in a class A laminar air flow cabinet or in a class B safety cabinet. Media or apparatus were sterilized routinely with an autoclave at 121°C for 15 min, though a longer autoclaving time was applied to large objects. Heat labile solutions were filtered through 0.45 μm sterile membranes into pre-sterilized receptacles.

2.2 Field experiment

2.21 Sampling of soil gas

Portable sub-surface gas monitoring probes were modified from Department of the Environment (UK) (1994) to collect soil gas under field conditions. Each was made from a steel pipe, 65 cm long, 2.5 cm outer diameter, 1.5 cm internal diameter. The lower 15 cm of the probe was perforated for gas movement between soil and the internal of the sampler. The probes were inserted into soil to 50 cm deep by a hammer for gas sampling; therefore, all results refer to the gas content at a depth of 35 to 50 cm from soil level. A gas sample was collected about 5 minutes after a sub-surface probe was inserted into topsoil. It was to purge the air from the outlet of a sub-surface probe 80 - 100 times by a 30- ml hand pump into an Alltech 150-ml glass gas bulbs made of glass or polypropylene and with Teflon stopcocks. Gas samples were transported to the laboratory and quantified by gas chromatography within 24 h (2.31).

2.22 Soil analysis

The bulk density of landfill sites topsoil was determined by the core method (Blake & Hartge, 1986). The core was made from stainless steel tube and the effective size was 10 × 4.8 cm internal diameter. Five random sampling points were studied on each site. Soil samples collected by the cores were stored in pre-weighted polycarbonate vessels (Magenta GA-7 vessel, from Sigma, USA) for further laboratory analysis (2.32). The laboratory analysis of bulk density also provided data for the moisture contents of soil samples.

Soil samples, next to the points for bulk density analysis, were collected for pH and extractable nitrogen analyses. The top 1 cm of cover soil was removed and samples were collected with a hand spade and stored in polystyrene bottles for subsequent chemical analyses (2.32).

2.23 Evaluation of tree cover

The abundance (number of individuals) and tree cover of perennial trees were counted and estimated according to Mueller-Dombois and Ellenberg (1974) and Westman (1985). Only trees higher than 1.3 m (breast height) were considered as mature and were counted (Goldsmith & Harrison, 1976). When the number of trees for a particular species was more than 20 on a site, the total umbers of trees were estimated in increments of 10. When the number was higher than 100, the whole site was divided into 5 to 10 districts and the tree number within each area was estimated. Tree cover was rated visually on the site. Maps and air photographs provided by the Land and Survey Department, Hong Kong, helped the map drawing and in estimating tree copy. Five-point Braun-Blanquet cover-abundance scale was adopted to indicate the spatial pattern of tree cover on sites and was illustrated on maps after field survey:

5	any number, with cover > 75% of the reference area
4	any number, with 50 - 75% cover
3	any number, with 25 - 50% cover
2	any number, with 5 - 25% cover
1	numerous, but less than 5% cover, or scattered, with cover up to 5%
+	few, with small cover
r	solitary, with small cover

2.3 Chemical and biochemical analysis

2.31 Analysis of landfill gas

The volume percentages N_2, CH_4, O_2 and CO_2 and methane on a sample were quantified with a gas chromatograph (GC), Hewlett Packard 5890 Series II, installed with a CTR I gas column from Alltech (H.K.) Ltd and detected by a thermal conductivity detector (TCD) (Alltech, 1989; Lodge, 1989). The use of a gas chromatograph is the most reliable current method for analysing the major components of landfill gas. It is also the only method of measuring the concentration of N_2 in a gas sample (Department of the Environment (UK), 1994). As the performance of the CTR I column in the preliminary study was not satisfactory, a 1 m × 0.468 cm (1/8 inch) stainless steel column packed with Porapak N (Alltech 2739) was connected before the CTR I column to increase its plate-count. Table 2.1 lists the configuration of the gas chromatograph for the separation of the gases. The suppliers of compressed gases in cylinders and the purities of the gases are tabulated in Table 2.2.

Table 2.1 Configuration of gas chromatograph for the separation of N_2, CH_4, O_2 and CO_2.

injector, oven and detector temperature	:	ambient
carrier gas flow	:	75 ml min^{-1} (He)
volume of injection	:	0.50 ml per sample
standard gas	:	Alltech multi-component gas mixture (Alltech 9799, Table 2.2)
standardization	:	three points linear: Alltech 9799, ambient air and instrument zero.

Table 2.2 Suppliers and purity of experimental gases.

compound/ element	supplier	purity ($\% \ v \ v^{-1}$)	remarks
H_2	Hong Kong Oxygen & Acetylene Co. Ltd	99.999	
He	"ditto"	99.999	
O_2	"ditto"	99.99	
Ar	Chun Wang Gas Co. Ltd	99.99	
CH_4	Hong Kong Oxygen & Acetylene Co. Ltd	99.99	
C_2H_2	"ditto"	instrument grade	
C_2H_4	Alltech (H.K.) Ltd	100 95.4 ppm	Alltech 714 Alltech G0411 (in He)
CO_2	Hong Kong Oxygen & Acetylene Co. Ltd	99.999	
compressed air	"ditto"		0.001% moisture
mixed standard	Alltech (H.K.) Ltd		Alltech 9799 (in N_2)
CO_2		15	
CO		7	
O_2		7	
CH_4		4.5	

The peak heights of chromatograms were integrated using a software package, ChemStation, version A.02.12, developed by Hewlett Packard (HP 3365).

Gas samples between 1.0 to 10.0 ml were transferred by a Hamilton 1000 series 10-ml gas-tight syringe graduated to 0.1 ml. A 1-ml gas-tight syringe of the same series, graduated to 0.02 ml, was employed to transfer samples between 0.1 to 1.0 ml. Teflon luer-lock detachable needles, point style #5 side-hole, gauge number 22s (Alltech 90238, were used with the above syringes for injection.

2.32 Analysis of biological, soil and leachate samples

2.321 Sample preparation

The sulphuric acid - hydrogen peroxide wet oxidation method, described by Allen (1989a & b), was used to prepare plant and soil samples. For plant samples, about 0.1 to 0.4 g of samples, measured to 0.1 mg accuracy, were digested in 4.4 ml mixed digestion reagent and the final volume was 25 ml. For soil samples, the mass of samples was restricted to below 0.2 g. The results of subsequent analysis of samples prepared by this wet oxidation method refer to the total composition of the samples.

Extractable portions of soil samples were prepared by extracting samples in KCl solution. About 10 g of fresh sample was extracted on 100 ml of 2 M KCl solution, shaken at about 100 rpm for 1 h and filtered through Whatman No. 42 filter paper. Samples were refrigerated at 4°C before the quantitative analyses (Allen, 1989a; Keeney & Nelson, 1982).

To digest liquid samples for metal analysis, 50 ml of sample was digested with 70% nitric acid at 483 KPa (70 p.s.i.) for 10 min in a CEM microwave oven, model MDS-2000.

2.322 Quantitative analysis of prepared samples

The soil pH of freshly collected sample was measured by half filling a 100-ml beaker with the sample and distilled water was added to full. The mixture was stirred thoroughly and stood for at least 10 min before measurement (Grimshaw, 1989).

The extractable NH_4-N in soil was analyzed by the indophenol blue method by a flow injection autoanalyzer, QuikChem Model AE, followed the QuikChem Method No. 30-107-06-1-A (Allen, 1989b; Keeney & Nelson, 1982; Knowles et al., 1989; Selmer-Olsen, 1971). The extractable NO_2–N and NO_3–N in soil samples were analyzed by the above flow injection autoanalyzer followed the QuikChem Method No. 30-107-04-1-A (Allen, 1989a & b; Keeney & Nelson, 1982).

The indophenol blue method was employed to determine the total Kjeldahl N in acid digested samples, using by a spectrophotometer at 625 nm (Grimshaw et al., 1989). The total Kjeldahl P content of acid digested samples were determined by the molybdenum blue method, using by a spectrophotometer at 700 nm (Grimshaw et al., 1989; Olsen & Sommers, 1982).

The Biochemical Oxygen Demand (BOD) of leachate was measured according to the American Public Health Association (1985). The osmotic pressure of leachate was measured at a vapour pressure osmometer Wescor Cor (USA) Model 5500.

Aqueous extracts of a soil sample were prepared by shaking 0.9950 to 1.0050 g of air dried soil in 50 ml distilled water at about 250 rpm for 2 h (McNeal, 1981; Phoades & Miyamoto, 1990). The conductivity of the aqueous extract was measured using a Philips conductivity meter Model PW 9526 with a 1-cm probe.

2.4 Media and culture techniques

2.41 Isolation and culture of rhizobia

2.411 Isolation of rhizobia

Pure rhizobia cultures for Acacia confusa and Leucaena leucocephala were isolated from the Junk Bay Landfill (Stage I). In the field, top soil adjacent to a legume tree was dug up with a 15-cm hand spade and inspected for turgid nodules with great care. Healthy pink nodules were collected together with some intact roots and soil and stored inside plastic bags chilled inside a chest box (10 - 15°C). Although nodules can be stored in a freezer or in a desiccator for a few weeks before isolation (Weaver & Frederick, 1982), isolation work was completed within 24 h.

The isolation procedures for rhizobia followed mainly Weaver and Frederick (1982) and Vincent (1970). In the laboratory, nodules were washed with sterile water and attached roots were picked off. Cleaned nodules were surface sterilised firstly in 70% ethanol, then in 0.1% acidified $HgCl_2$ solution for 1 - 3 min (disinfection period depends on size and source of nodule sample) and finally rinsed in sterile water. To collect viable rhizobia cells, surface sterilized nodules were squashed on a Petri dish by a glass rod and the cell sap was then streaked on yeast-mannitol agar plates (YMA) (Table 2.3) and incubated at 24 - 30°C. Rhizobial colonies appeared within 2 - 4 days, depending on the physiological state of the rhizobia at the time of plating; sizeable colonies which appeared within 1 or 2 days were not rhizobia. Rhizobial colonies, well clear of other, somewhat translucent, circular, raised on the agar surface and relatively odourless (except for the normal odours associated with the medium) were subcultured on YMA plates.

To further isolate rhizobia from other bacteria, mixed colonies were streaked on YMA-Congo red medium (Table 2.4). Many bacteria take up this Congo red dye and their colonies appear red, but rhizobia do not absorb the dye.

The rhizobia were further separated from other bacteria before a pure culture was established. Mixed cultures were suspended in yeast-mannitol broth (YMB) (Table 2.3) and shaken for 3 - 4 days at 28 - 30°C. Suspension cultures were then diluted into several concentrations of YMB, mixed well and reinoculated on YMA. Plates with well-isolated colonies were used to establish a pure culture.

2.412 Authentication studies

Rhizobia were separated from Agrobacterium spp. by the NaCl-tolerance test (Weaver & Frederick, 1982). This step was essential as Agrobacterium spp. can form root gall which cannot fix N_2. Agrobacterium spp. can tolerate 2% NaCl at pH 4.4, but not rhizobia. Cultures were inoculated on YMA-NaCl plates (Table 2.4) in a series of 1, 4, 10, 14 and 20 g NaCl l^{-1} and incubated at 28 - 30°C. Those cultures tolerating 2% NaCl were not rhizobia.

The ability to nodulate a legume remains the final arbiter as to a culture's allocation to the genus Rhizobium. The basic experimental requirement is that seeds of the test host, the substrate and the containers are free of rhizobia. Before the infection test, rhizobia from the culture stock were subcultured on YMB. After 4 d, there should be 10^7 to 10^9 cells ml^{-1} in the subculture (2.42). Then, 1 ml suspension culture was inoculated on rhizobia-free plants (2.5). If the culture was rhizobia, nodules would form on host roots and could be visually identified under a stereo microscope shortly one week after the inoculation. Final confirmation of ability to fix N_2 as shown by the acetylene reduction assay (2.7) could be conducted only after another month, when the nodules were mature.

2.42 Microbe density

To measure the density of the rhizobia culture, samples were diluted in a ten-fold serial dilution of YMB and incubated at 37°C for 3 d. The density was counted on a colony counter and expressed as cells ml^{-1}.

Table 2.3 Chemical composition of YMA/YMB. Adjust pH to 6.8 - 7.0 with 1 M HCl before autoclaving. Omit agar to prepare YMB.

Compound/ element	molecular/ atomic weight	stock ($g\ l^{-1}$)	medium ($mg\ l^{-1}$)	medium (μM)	total element ($mg\ l^{-1}$)	total element (μM)
$MgSO_4 \cdot 7H_2O$	246.47	2.00	200	811		
Mg	24.305		19.7		19.7	811
S	32.060		26.0		26.0	811
NaCl	58.44	1.00	100	1711		
Na	22.990		39.3		39.3	1709
Cl	35.453		60.7		60.8	1715
K_2HPO_4	174.18	4.00	400	2296		
K	39.098		179.6		179.6	4594
P	30.974		71.1		71.1	2295
$Na_2MoO_4 \cdot 2H_2O$	241.95	0.0200	0.0200	0.083		
Na	22.990		0.0038			
Mo	54.938		0.0045		0.0045	0.082
$MnSO_4 \cdot H_20$	169.01	0.200	0.200	1.183		
Mn	54.938		0.0650		0.0650	1.183
S	32.06		0.0379			
H_3BO_3	61.83	0.0200	0.200	0.323		
B	10.810		0.0035		0.323	29.9
$FeCl_3$	162.21	0.400	0.400	2.466		
Fe	55.847		0.0344		0.0344	0.616
Cl	35.453		0.0874			
$CaCO_3$	100.09	(freshly	200.0	1998		
Ca	40.080	prepare)	80.1		80.1	1996
C	12.011		24.0		24.0	1998
mannitol		ditto	10000			
yeast extract		ditto	400			
agar		ditto	15000			

Table 2.4 Composition and preparation of YMA-Congo red and YMB-NaCl. Adjust pH to 6.8 - 7.0 with 1 M HCl before autoclaving. Omit congo red and adjust NaCl to 1 - 20 g l^{-1} to prepare YMB-NaCl.

Compound/ element	molecular/ atomic weight	stock ($g\ l^{-1}$)	medium ($mg\ l^{-1}$)	medium (μM)	total element ($mg\ l^{-1}$)	total element (μM)
$MgSO_4 \cdot 7H_2O$	246.47	2.00	200.0	811		
Mg	24.305		19.7		19.7	811
S	32.060		26.0		26.0	811
NaCl	58.44	1.00	100.0	1711		
Na	22.990		39.3		39.3	1709
Cl	35.453		60.7		60.8	1715
K_2HPO_4	174.18	4.00	400	2296		
K	39.098		179.6		179.6	4594
P	30.974		71.1		71.1	2295
$Na_2MoO_4 \cdot 2H_2O$	241.95	0.0200	0.020	0.083		
Na	22.990		0.0038			
Mo	54.938		0.0045		0.0045	0.082
$MnSO_4 \cdot H_20$	169.01	0.200	0.200	1.183		
Mn	54.938		0.0650		0.0650	1.183
S	32.06		0.0379			
H_3BO_3	61.83	0.0200	0.200	0.323		
B	10.810		0.0035		0.323	29.9
$FeCl_3$	162.21	0.400	0.400	2.466		
Fe	55.847		0.0344		0.0344	0.616
Cl	35.453		0.0874			
$CaCO_3$	100.09	(freshly	200.0	1998		
Ca	40.080	prepare)	80.1		80.1	1996
C	12.011		24.0		24.0	1998
congo red		ditto	2500			
mannitol		ditto	10000			
yeast extract		ditto	400			
agar		ditto	15000			

2.5 Plant care

2.51 Source of seeds and seedlings

Leucaena leucocephala seeds were collected from the Junk Bay Landfill (Stage I). Acacia confusa seeds were collected from Shatin along Lion Rock Tunnel Road near Tsang Tai Uk (not a landfill site). All seeds were dried under direct sunshine and stored in a desiccator with silica gel desiccant.

One-year old Cinnamomum burmanii and Tristania conferta (non-legumes) were supplied by the Tai Tong Nursery, Hong Kong Government.

2.52 Substratum

Agar, perlite and vermiculite are common substrata for rhizobial infection studies. However, in order to have a substratum which allows gas to diffuse freely, with high water holding capacity as well as to ease the work in isolating nodules for ARA, vermiculite was chosen. Moreover, to maximize gas movement, only vermiculite granules larger than 2.0 mm (passing through a stainless steel sieve) were used for planting. As commercial vermiculite may be contaminated with rhizobia, all vermiculite granules were washed three times with tap water and autoclaved for 30 min before use.

2.53 Seed germination

The hard seed coats of Acacia confusa and Leucaena leucocephala were digested partially in acid before germination. The seeds were soaked in concentrated H_2SO_4 for 1 min, and then rinsed in ice chilled water. The use of cold water was to avoid the damage on seeds by the heat generated when the acid was mixed with water. Acid-treated seeds were further disinfected in 0.1% acidified $HgCl_2$ solution for 2 min and rinsed with sterile distilled water. Disinfected seeds were germinated in wet vermiculite and in the dark at 22 - 28°C. Viable seeds formed young roots within 3 d.

2.54 Care of seedlings

Various containers were employed to hold the seedlings for different experiments: these included 4 cm (diameter) × 5 cm (tall) low density polypropylene pots (for the short-term assay in Chapter 4), polythene seedling bags (10 cm (diameter) × 10 cm (tall), for Chapters 5 and 6) and fumigation chambers (for the long-term assay in Chapter 4). Fumigation chambers were modified from 500-ml low density polypropylene laboratory wash bottles (Nalgene 2402-0500) and had a spray tube molded on the side wall. To avoid rhizobia contamination, all chambers were surface sterilized in 5% Clorox solution for 15 min and were rinsed three times with sterile distilled water before filling with sterile vermiculite. To prepare seedlings for the long-term assay in Chapter 4, legume seeds were firstly germinated as described in Section 2.53. Then, young seedlings were transplanted to a fumigation chamber within two weeks after germination. Each chamber held three seedlings of the same species.

Seedlings in fumigation chambers were cultured inside an air-conditioned growth room preset at 24 - 26°C and with a relative humidity around 80%. Philips series TLD fluorescent lamps for horticulture and Philips series MLR blended-light lamps (red spectrum enhanced) were used and 900 µmol m^{-2} s^{-1} PAR was achieved. The photoperiod was set to 16-h-light and 8-h-dark with a 24-h timer. Other seedlings in seedling bags and pots were kept at a greenhouse with plant-light enhanced illumination. The actual irradiance varied, depended on the solar radiation.

Two culture solutions for plants were used: N-free (Table 2.5), N-enriched (Table 2.6). Their application rates on seedlings varied on different experiments.

Table 2.5 Mineral salt composition of N-free culture medium for woody plants, pH 6.5.

Compound/ element	molecular/ atomic weight	stock ($g\ l^{-1}$)	medium ($mg\ l^{-1}$)	medium (μM)	total element ($mg\ l^{-1}$)	total element (μM)
K_2SO_4	136.14	21.74	348.0	2556		
K	39.098		199.9		199.9	5113
S	32.060		82.0		264.5	8250
$NaH_2PO_4 \cdot 2H_20$	156.01	46.00	202.4	1297		
Na	23.990		31.1		33.0	1377
P	30.974		40.2		40.2	1298
$MgSO_4 \cdot 7H_20$	246.47	46.0	368.0	1493		
Mg	24.305		36.3		36.3	1494
S	32.060		47.9			
$CaSO_4 \cdot 2H_20$	172.17	80.0	720.0	4182		
Ca	40.080		167.6		167.6	4182
S	32.060		134.1			
$FeCl_3$	162.21	3.30	13.2	101		
Fe	55.847		4.54		4.54	81.3
Cl	35.453		8.66		11.59	36.9
$MnSO_4 \cdot H_20$	169.01	2.30	2.300	13.61		
Mn	54.938		0.748		0.748	13.6
S	32.060		0.436			
$ZnSO_4 \cdot 7H_20$	287.54	0.290	0.290	1.009		
Zn	65.380		0.066		0.066	1.0
S	32.060		0.032			
$CuSO_4 \cdot 5H_20$	249.68	0.240	0.240	0.967		
Cu	63.546		0.061		0.061	1.0
S	32.060		0.031			
H_3BO_3	61.83	3.10	3.100	50.14		
B	10.810		0.542		0.542	50.1
$Na_2MoO_4 \cdot 2H_2O$	241.95	0.120	0.120	0.496		
Na	22.990		0.023			
Mo	54.938		0.027		0.027	0.5
NaCl	58.44	4.84	4.840	82.82		
Na	22.990		1.904			
Cl	35.453		2.936			
$CoSO_4 \cdot 7H_2O$	281.10	0.046	0.046	0.164		
Co	58.933		0.010		0.010	0.2
S	32.060		0.005			

Table 2.6 Mineral salt composition of N-enriched culture solution for woody plants, pH 6.5.

Compound/ element	molecular atomic weight	stock ($g\ l^{-1}$)	medium ($mg\ l^{-1}$)	(μM)	total element ($mg\ l^{-1}$)	(μM)
KNO_3	101.10	40.60	324.8	3213		
K	39.098		125.6		125.6	3212
N	14.007		45.0		121.1	8646
$Ca(NO_3)_2$	236.15	80.24	641.9	2718		
Ca	40.080		108.9		108.9	2717
N	14.007		76.1			
$MgSO_4 \cdot 7H_20$	246.47	46.0	368.0	1493		
Mg	24.305		36.3		36.3	1494
S	32.060		47.9		48.4	1510
$NaH_2PO_4 \cdot 2H_20$	156.01	46.0	202.4	1297		
Na	23.990		31.1		33.0	1376
P	30.974		40.2		40.2	1298
$FeCl_3$	162.21	3.30	13.2	101		
Fe	55.847		4.54		4.54	81.3
Cl	35.453		8.66		11.59	36.9
$MnSO_4 \cdot H_20$	169.01	2.30	2.30	13.61		
Mn	54.938		0.748		0.748	13.6
S	32.06		0.436			
$ZnSO_4 \cdot 7H_20$	287.54	0.290	0.290	1.009		
Zn	65.380		0.066		0.066	1.0
S	32.060		0.032			
$CuSO_4 \cdot 5H_20$	249.68	0.240	0.240	0.967		
Cu	63.546		0.061		0.061	1.0
S	32.060		0.031			
H_3BO_3	61.83	3.10	3.100	50.14		
B	10.810		0.542		0.542	50.1
$Na_2MoO_4 \cdot 2H_2O$	241.95	0.120	0.120	0.496		
Na	22.990		0.023		0.027	0.5
Mo	54.938		0.027			
NaCl	58.44	4.84	4.84	82.82		
Na	22.990		1.904			
Cl	35.453		2.936			
$CoSO_4 \cdot 7H_2O$	281.10	0.046	0.046	0.164		
Co	58.933		0.010		0.010	0.2
S	32.060		0.005			

2.6 Preparation of gases for fumigation test

Simulated landfill gas was obtained from anaerobic bio-digestion of organic materials (Table 2.7). As the degradation process of the packing materials was similar to the degradation process of domestic waste, the gas from a digester was similar to the chemical composition of landfill gas. Each digester produced about 10 l d^{-1} gas and lasted for about a month. When the simulated landfill gas was fed into a fumigation chamber (2.54) via the opening of its spray tube, the gas diffused from the bottom of the chamber, passed through the vermiculite and emitted via the opening at the top. On its way and inside the chamber, the gas fumigated the root portion of plants growing on the vermiculite.

Table 2.7 Preparation of digester for the production of simulated landfill gas.

component	f. wt (kg)	d. wt (kg)	remarks
pig manure, fresh	0.30	0.036	% contents: C = 40, N = 4
pig manure, oven dried	0.20	0.194	To lower the viscosity during handling.
steamed rice	0.30	0.153	To increase the C content to 35%, optimal range is 20 to 30 (Wong, 1987).
seeding	0.10	0.015	From old digester, to provide adequate methanogen inocula.
soy bean waste	0.30	0.113	
sodium buffered tap water (pH 7.5)	5.00		When the pH drops below 6.6, there is significant inhibition of the CH_4-producing bacteria, and a pH of 6.2 is toxic to them (Hawkes, 1979; Ruskin, 1982). Optimal moisture content for digestion is about 85 to 90%.
total	6.20	0.511	91.8% moisture

CO_2 supplied by the Hong Kong Acetylene and Oxygen Co. (Table 2.2) was used to assay the influence of landfill gas. For the long-term assay in Chapter 4, an electrical gas control panel was fabricated to ensure the CO_2 from cylinder (13800 kPa = 2000 p.s.i) passed constantly and evenly to each fumigation chamber (4.31). The control panel had one inlet and six outlets. CO_2 from the cylinder was diverted from the inlet to the different outlets in sequence, controlled by solenoid valves. Each cycle was 1 min and the flow rate from cylinder was adjusted to 160 ml min^{-1}. Therefore, 20 ml min^{-1} of CO_2 was passed into each fumigation chamber.

2.7 Photosynthesis

PP Systems model CIRAS-1 photosynthesis system with a Pakinson leaf cuvette was used to measure the leaf photosynthetic rate of tree seedlings; ambient air was fed into the machine and the deviation in CO_2 concentration after passing the cuvette was measured. Quantum flux density during measurement was controlled by the CRS061 light unit and measurement was made under laboratory conditions: about 22°C, RH 60%. All testing was conducted after 10:00 in the morning and before 16:00 in the afternoon.

2.8 Acetylene reduction assay

The acetylene reduction assay (ARA) procedure was developed as described by Criswell et al. (1976), Knowles (1986), Masterson and Murphy (1980) and Turner and Gibson (1980). Preliminary studies showed the C_2H_4 levels could not be detected by the gas chromatograph when intact plants and 2.5 l incubation vessel were used (0.03 to 0.05 g nodule (f. wt) $plant^{-1}$); therefore, nodulated roots were used throughout the whole project.

Nodule samples were keep inside a 35-ml McCartney glass bottle and sealed with a blood serum stopper for the ARA. The stoppers were pre-wetted with water before inserted into the bottle; this maintained the sample moisture and ensured the bottle was air-tight. When ARA was conducted at ambient conditions, the pressure inside a bottle was first adjusted to 0.95 atm. 2 × 1.00 ml air was withdrawn from a sealed bottle before acetylene injection. When 1.75 ml instrument grade acetylene (Table 2.2) was injected into the bottle, the pressure was restored to 1.00 atm (the actual O_2 concentration in a bottle was 19.8%). The incubation period was 1 h, unless specified. All analyses were conducted in an air-conditioned laboratory, where the temperature was maintained at 21°C.

When it was necessary to adjust to the O_2 to a series of fixed concentrations (Chapter 4), a 35-ml McCartney glass bottle with decapitated nodules was first purged with Ar at 5 l min^{-1} for 30 s. Two needles was inserted into the stopper, one for passing Ar from cylinder into the bottle and the other for venting. A 10 - 15 times exchange of gas was suggested to replace all the ambient air in a reaction bottle for ARA (Turner & Gibson, 1980); purging in the above way has already exchanged the bottle gas content about 70 times. Although King and Layzell (1991) reported that the presence of Ar might cause a decline in nodule gas permeability, Ar is still the better gas, in comparison to other gases, to be used as a balance gas to set up a O_2 gradient. After purging with Ar, the gas inside the bottle was evacuated as described above so that after the addition of O_2 and acetylene, the pressure of the bottle was restored to 1.00 atm.

The ethylene concentrations in bottles were determined by a Hewlett Packard 5890 series II gas chromatograph. Peak heights were integrated using the software package, ChemStation, developed by Hewlett Packard. The details of the hardware configurations are listed in Table 2.8. The detection limit of the above system was about 1 μmol C_2H_4.

Table 2.8 Configuration of the gas chromatograph for the determination of ARA.

dectector	:	H_2 flame ionization detector (FID); H_2, 25 ml min^{-1}; air, 200 ml min^{-1} (138 kPa (20 p.s.i.)); temperature 50°C
column	:	stainless steel, 1 m x 1/8 inch internal diameter; Porapak S (80/100 mesh)
carrier gas	:	N_2, 414 kPa (60 p.s.i.), 35 ml min^{-1}
oven temperature	:	isothermal, 50°C
injector temperature	:	50°C
septum type	:	tri-layer
retention time	:	C_2H_4, 0.7 min; C_2H_2, 0.8 min

2.9 Computing

80386 or above PC machines were made available in the University of Durham. Text and tables were edited by Microsoft Word version 2.0. Graphs were generated by SigmaPlot for Windows, version 1.01, by Jandel Scientific. Data were organized by Microsoft Excel version 3.0. Statistical analyses, including mean, SD, ANOVA and correlation were computed by built-in functions of Excel. Two software packages were used to draw the maps: Publisher's Paintbrush, version 2.00, developed by ZSoft Corp, Paintbrush, version 3.0, developed by Microsoft Corp.

CHAPTER 3
FIELD SURVEY

3.1 Introduction

Although there is much information on tree growth on temperate landfill sites (1.17), similar information for tropical and subtropical sites is relatively rare (1.13). In most Far East cities, landfill is one of the major methods of waste disposal (1.17); therefore, there is an urgent need to seek tolerant species to revegetate completed sites. Leguminous trees are known to be suited to grow on subtropical completed sites (1.133); however, better understanding can be obtained only when the growth and performance of legumes and non-legumes are compared under actual landfill conditions. For this purpose, a tree survey was conducted in 13 landfill sites in Hong Kong. The growth of legumes and non-legumes, based on tree number and percent tree cover on sites, was compared.

3.2 Method

All the 13 completed or on-going landfill sites were surveyed between September 1993 and March 1994. This is the dry season in Hong Kong; soil samples were not collected if there had been rain within two days.

For each species, the total number of trees and relative percentage tree cover of mature tress ($\geq$ 1.3m tall) (2.23) on each site were recorded. Then, the overall relative percent tree cover of each species was calculated. Gas samples at the landfill topsoil were collected at five random points on each site by sub-surface probes (2.21) and the major components of the samples were determined (2.31). The sampling points were at least 5 m apart, accessible and located generally on the 0.25, 0.5 and 0.75 of two diagonal lines of a site. The bulk densities of topsoil were analyzed on five similar points on each site. Soil samples within 0.5 m from the points of the bulk density test were collected for physical and chemical analysis (2.22).

3.3 Soil conditions

The mean CO_2 concentrations collected by sub-surface probes ranged from 0.03 to 17.4%, while the O_2 concentrations ranged from 10.3 to 20.9% (Table 3.1). Methane was detected at five sites and ranged from < 0.1 to 28.2%.

The landfill gas levels at new sites were high. In the on-going Shuen Wan Landfill, high CO_2 (mean = 3.76%, SD = 4.32%) and high CH_4 (mean = 4.18%, SD = 3.81%) were measured. In contrast, the landfill gas levels at old sites were low. For example, in the Siu Lang Shui Landfill, which has been closed for 11 years, the mean CO_2 concentration was 0.08% (SD = 0.04%) and the CH_4 concentration was below the detection limit (≅ 0.005%). However, some old sites also had high landfill gas levels. Among the 13 sites, the highest landfill gas level was measured on an old site: the Ma Tso Lung Landfill (completed on 1979) (mean CO_2 = 17.4%, SD = 14.4%; mean CH_4 = 28.2%, SD = 24.1%). In this site, about 90% of the land was covered by sawdust waste when the gas sample was collected. In contrast, some new sites also had low landfill gas levels in topsoil. For example, Ma Yau Tong Central Landfill was closed for eight years, its CO_2 concentrations were relatively low (mean = 0.08%, SD = 0.03%) and CH_4 was non-detectable. The presence of extensive above-ground gas vent pipes, probably the highest pipe density among the 13 sites, was a unique feature of the site. The Pillar Point Landfill was an on-going site, a low concentration of landfill gas was measured (CO_2 mean = 0.05%, SD = 0.03%).

The pH of the topsoil at most sites was generally acidic and ringing from 4.34 - 6.89, except Pillar Point (pH 7.38) (Table 3.2). The bulk densities of eight sites were higher than 1.40 g cm^{-3} (maximum = 1.87) while others were ranged from 1.00 - 1.34 g cm^{-3}. Except for the Ma Tso Lung Landfill, where most of the land was covered by sawdust, the moisture content of the topsoil at all other sites was low, ranging from 8.8 - 16.8%. Waterlogging was restricted to certain edge areas. These were < 10 m^2 and seen only after heavy rain.

The extractable NH_4-N in the topsoil ranged from 213 - 1400 μg NH_4-N 100 g^{-1} (d. wt), while the NO_2-N and the NO_3-N ranged from 15 to 168 μg NO_2-N 100 g^{-1} (d. wt) and 7.8 to 30.3 μg NO_3-N 100 g^{-1} (d. wt).

Table 3.1 Gaseous composition (% v v^{-1}) in landfill topsoil at the 13 sites. All units are in % v v^{-1}, ND = not detectable (≤ 0.01%), n = 5.

		Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers' Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
CO_2	mean	0.58	0.23	0.030	17.4	0.47	0.17	0.08	0.08	0.53	3.76	11.29	7.90	0.05
	SD	0.96	0.26	0.002	14.4	0.57	0.14	0.04	0.03	1.56	4.32	11.44	9.94	0.03
CH_4	mean	ND	ND	ND	28.2	ND	ND	ND	ND	<0.1	4.18	11.7	17.5	ND
	SD				24.1						3.81	14.7	14.0	
O_2	mean	20.5	20.3	20.7	10.3	20.6	19.9	20.6	20.5	19.8	16.8	10.9	15.4	20.9
	SD	0.7	0.2	0.2	8.7	0.5	0.5	0.2	0.2	1.6	4.4	9.02	7.3	0.1
N_2	mean	78.1	75.6	74.6	44.5	78.0	72.9	76.9	75.7	76.9	73.7	65.4	61.3	78.1
	SD	0.8	1.1	1.4	28.7	0.0	1.9	0.6	0.7	0.4	9.3	19.6	21.6	0.5

Table 3.2 Chemical properties of topsoil at the 13 landfill sites.

variable		Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
pH		5.34	5.29	6.89	4.34	6.59	5.88	6.25	5.48	5.14	6.57	4.68	5.93	7.38
bulk density (g cm^{-3})	mean	1.49	1.25	1.87	1.27	1.45	1.50	1.00	1.41	1.34	1.87	1.31	1.42	1.42
	SD	0.11	0.07	0.12	0.3	0.04	0.09	2.0	0.11	0.13	0.07	0.16	0.23	0.18
soil moisture (%)	mean	15.6	13.4	8.31	21.5	8.8	12.5	15.3	16.8	12.6	7.5	14.8	12.8	15.3
	SD	1.7	1.0	0.78	6.3	2.8	1.8	4.3	4.6	1.8	0.57	2.3	3.2	4.3
extractable-NH_3	mean	279	371	1240	523	222	231	327	498	340	1400	213	404	498
(µg NH_4-N 100 g^{-1} (d. wt))	SD	238	217	0.31	369	100	85	116	190	71	350	137	207	132
extractable-NO_2	mean	168	15.0	28.5	60.2	19.8	20.9	222	166	25.2	26.2	84.8	122	110
(µg NO_2-N 100 g^{-1} (d. wt))	SD	303	3.8	28.8	14.4	11.6	10.4	220	268	19.1	22.8	25.7	108	106
extractable-NO_3	mean	12.5	9.0	14.5	17.3	12.1	7.8	9.0	13.0	10.0	14.6	9.7	15.5	30.3
(µg NO_3-N 100 g^{-1} (d. wt))	SD	6.1	7.0	2.1	13.1	6.1	3.3	5.3	3.8	6.0	13.9	6.5	9.8	28.1

3.4 Tree cover at the 13 sites

The numbers of tree (Table 3.3), tree cover (Table 3.4) and other unique characteristics of each of the 13 sites are described in ascending order of the closing date of the site.

Ngau Tam Mei

Although this site had been closed for nearly 20 years, it had only a low tree cover (1%). Trees were confined to the southern side slope areas and the top platform was bare (Fig. 3.1). Acacia confusa was the only legume on the site. A non-legume, Sapium discolor, was especially abundant (20 stands, relative cover = 70%). (Another single stand was found in Ma Yau Tong Central; the other 11 sites were free of this species.)

Ngau Chi Wan

When the survey was conducted, about 70% of the site had been covered with cement for coach and heavy vehicle parking. Beside the cemented platform areas which were not included in the survey, about 60% of land in the side slope was covered by trees (Fig. 3.2, Table 3.4). From the random location of trees and their unpruned growth, it was assumed that they had neither been planted deliberately nor with much care. Although the total area which allowed plant growth was small (2.46 ha, excluding cemented area), 12 species of tree were found. Three of these were legumes: Acacia confusa, Alysicarpus vaginalis, Bauhinia blakeana.

Gin Drinker's Bay

Although about 30 species of tree had been planted on this site (1.132), only 20 species were left (8 legumes, 12 non-legumes) (Table 3.3). Generally, the side slopes had above 70% tree cover, while the top platform had less than 5% tree cover (Fig 3.3). The total tree cover was 40%.

Ma Tso Lung

About 90% of this site was used for open dumping of sawdust waste; therefore, the site was almost completely bare of trees. Only one stand of legume (Acacia mangium) and one stand of non-legume (Lantana camara), probably self-seeded, were found on this small and remote site (2.0 ha) (Fig. 3.4).

Sai Tso Wan

This site had 45% tree cover (Fig. 3.5) and was mainly confined to three species: Acacia confusa (about 10000 trees), Eucalyptus citriodora (about 1000 trees), Leucaena leucocephala (about 100 trees) (Table 3.1). Apparently, the Acacia confusa was planted there initially along grid lines at a fixed distance to each other and formed a dense population with about 4 trees m^{-2}. The dense planting was presumably the cause of the stunted growth of the A. confusa (mostly < 3 m) and the exclusion of other species. Mature Eucalyptus citriodora, which has a straight trunk and is generally taller than 15 m, had become much taller than Acacia confusa. About 100 trees of Leucaena leucocephala were found.

Ma Yau Tong West

Although it has been proposed that the site should be converted to a park (1.15), it was simply an unused open space during the survey period and did not have much tree cover (5%). Trees were abundant at the top area and on the northern slope (Fig. 3.6). Acacia confusa had the highest relative cover (80%). Beside A. confusa, young seedlings (< 1.3 m tall) of another legume, Leucaena leucocephala, was scattered throughout the whole site.

Siu Lang Shui

This site had the highest percentage tree cover (90%) among the 13 sites (Fig. 3.7). About 18500 trees, belonging to seven species, were found. Three were legumes: Acacia confusa, A. mangium, Leucaena leucocephala (total relative cover = 66%).

Ma Yau Tong Central

This valley-fill site forms a bench and platform arrangement for tree growth (Fig. 3.8). Eleven species of trees were found, of which Acacia confusa and Leucaena leucocephala had 60% and 10% relative covers.

Jordan Valley

The tree cover was one of the lowest. Only 5% of the land was covered by trees and they were confined to the lower side-edge regions (Fig. 3.9). The relative cover of Leucaena leucocephala was the highest on this site (60%). Although it was a valley fill, the top platform was only about 20 m below the peak of the small hill which forms the valley. The top platform of the site was flat and extremely bare; only a 2 m tall Acacia mangium was found.

Shuen Wan

This was one of the four on-going sites. 80% of the site was bare of trees and actively in use for waste dumping (Fig 3.10). Seven species of tree were found on the completed and landscaped areas. Three were legumes: Acacia confusa, Albizzia lebbek, Leucaena leucocephala. A non-legume edible fruit tree was found: Psidium guajava.

Junk Bay Stage I

Junk Bay was an on-going marine-fill site. About 60% of the site area was bare of trees and in-use for waste dumping (Fig 3.11). On the remaining 40% of land, Acacia confusa, Casuarina equisetifolia and Eucalyptus torelliana were relatively abundant (relative covers 50%, 30%, 10%). In early March 1994, there was a small woodland of 40 mature and flower-bearing Bombax malabaricum, which had a maximum height of only 2 m.

Junk Bay Stage II/III

About 75% of this 35-ha landfill site was on-going in waste dumping and bare of trees (Fig. 3.12). The remaining 25% of land was also totally bare of mature trees.

Pillar Point Valley

This remote side had been operated for 10 years. About 65% of the site was on-going for waste dumping and bare of trees (Fig 3.13). Acacia confusa and Leucaena leucocephala were the most abundant species (relative covers = 60%, 15%).

Table 3.3 Total numbers of tree at the 13 landfill sites. [1] = legume family. All no. > 20 were estimated (2.23).

family	Latin name	Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers' Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
Anacardiaceae	Rhus succedanea		10											
Bombacaceae	Bombax malabaricum		1									40		
Caesalpiniaceae [1]	Bauhinia blakeana			10										
	Bauhinia purpurea			30										
	Cassia surattensis		1	100										
	Delonix regia			50										
	Peltophorum pterocarpum			50										
Capparidacea	Crataeva religlosa			20										
Casuarinaceae	Casuarina equisetifolia			100		10		1000		5		100		10
	Casuarina stricta							3000						
Euphorbiaceae	Aleurites moluccana			100										
	Aporusa chinensis		19				1		3			10		
	Breynia fruticosa		23											
	Macaranga tanarius	4	6	30		10	40		17	1	2			6
	Mallotus paniculatus			10			2		9					
	Sapium discolor	20							1					
	Sapium sebiferum								1		1			
Hammamelidaceae	Liquidambar formosana			20										
Lauraceae	Litsea glutinosa			100										
Magnoliaceae	Michelia mandiae								3					

(to be continued)

Table 3.3 (Continued)

family	latin name	Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers' Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
Melastomaceae	Melastoma sanguineum								7					
Mimosaceae[1]	Acacia auriculiformis							400						
	Acacia confusa	1	4	2000		10000	60	10000	200	9	48	300		50
	Acacia mangium				1			100		6				
	Albizzia lebbek			10							1			
	Leucaena leucocephala			100		100	70		59	32	49	10		20
Moraceae	Broussonetia papyrifera										1			
	Ficus microcarpa			10										
Myrtaceae	Eucalyptus citriodora					1000						8		
	Eucalyptus torelliana							4000				80		
	Psidium guajava									20	1			
	Tristania confusa		4	200				100						
Oleaceae	Ligustrum sinense								1					
Palmae	Archontophoenix alexandrae			10										
	Cocos spectabilis			10										
Papilionaceae[1]	Dalbergia benthami		1											
Rosaceae	Rhaphiolepis indica									1				
Sapindaceae	Euphoria longan								3					
Solanaceae	Solanum sp.		1											
Ulmaceae	Celtis sinensis	3	5	100			2			2	1			4
Verbenaceae	Lantana camara	4	21		1		30		5	2	1			

Table 3.4 Relative tree cover at the13 landfill sites. All units in %, [1] = legume family.

Family	Latin name	Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers' Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
Anacardiaceae	Rhus succedanea		10											
Bombacaceae	Bombax malabaricum		1									5		
Caesalpiniaceae [1]	Bauhinia blakeana			1										
	Bauhinia purpurea			1										
	Cassia surattensis		1	5										
	Delonix regia			2										
	Peltophorum pterocarpum			2										
Capparidaceae	Crataeva religlosa			1										
Casuarinaceae	Casuarina equisetifolia			5		1		5		5		30		10
	Casuarina stricta							14						
Euphorbiaceae	Aleurites moluccana			5										
	Aporusa chinensis		25				1		1			2		
	Breynia fruticosa		25											
	Macaranga tanarius	10	10	1		1	5		10	4	2			15
	Mallotus paniculatus			1			1		3					
	Sapium discolor	70							1					
	Sapium sebiferum										1			
Hammamelidaceae	Liquidambar formosana			1										
Lauraceae	Litsea glutinosa			1										
Magnoliaceae	Michelia mandiae								1					

(to be continued)

Table 3.4 (Continued)

Family	Latin name	Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan	Junk Bay Stage I	Junk Bay Stage II/III	Pillar Point Valley
Melastomaceae	Melastoma sanguineum								1					
Mimosaceae [1]	Acacia auriculiformis							5						
	Acacia confusa	5	13	50		94	80	50	60	15	70	50		50
	Acacia mangium				30					7				
	Albizzia lebbek			1							1			
	Leucaena leucocephala			10		1	10		20	60	22	2		15
Moraceae	Broussonetia papyrifera										1			
	Ficus microcarpa			1										
Myrtaceae	Eucalyptus citriodora					3						1		
	Eucalyptus torelliana							20				10		
	Psidium guajava										1			
	Tristania confusa		2	5				5		5				
Oleaceae	Ligustrum sinense								1					
Palmae	Archontophoenix alexandrae			1										
	Cocos spectabilis			1										
Papilionaceae [1]	Alysicarpus vaginalis		2											
Sapindaceae	Euphoria longan								1					
Scrophulariaceae	Paulownia fortunei													
Solanaceae	Solanum sp.		1											
Ulmaceae	Celtis sinensis	10	5	5			1			2	1			10
Verbenaceae	Lantana camara	5	5		70		2		1	2	1			

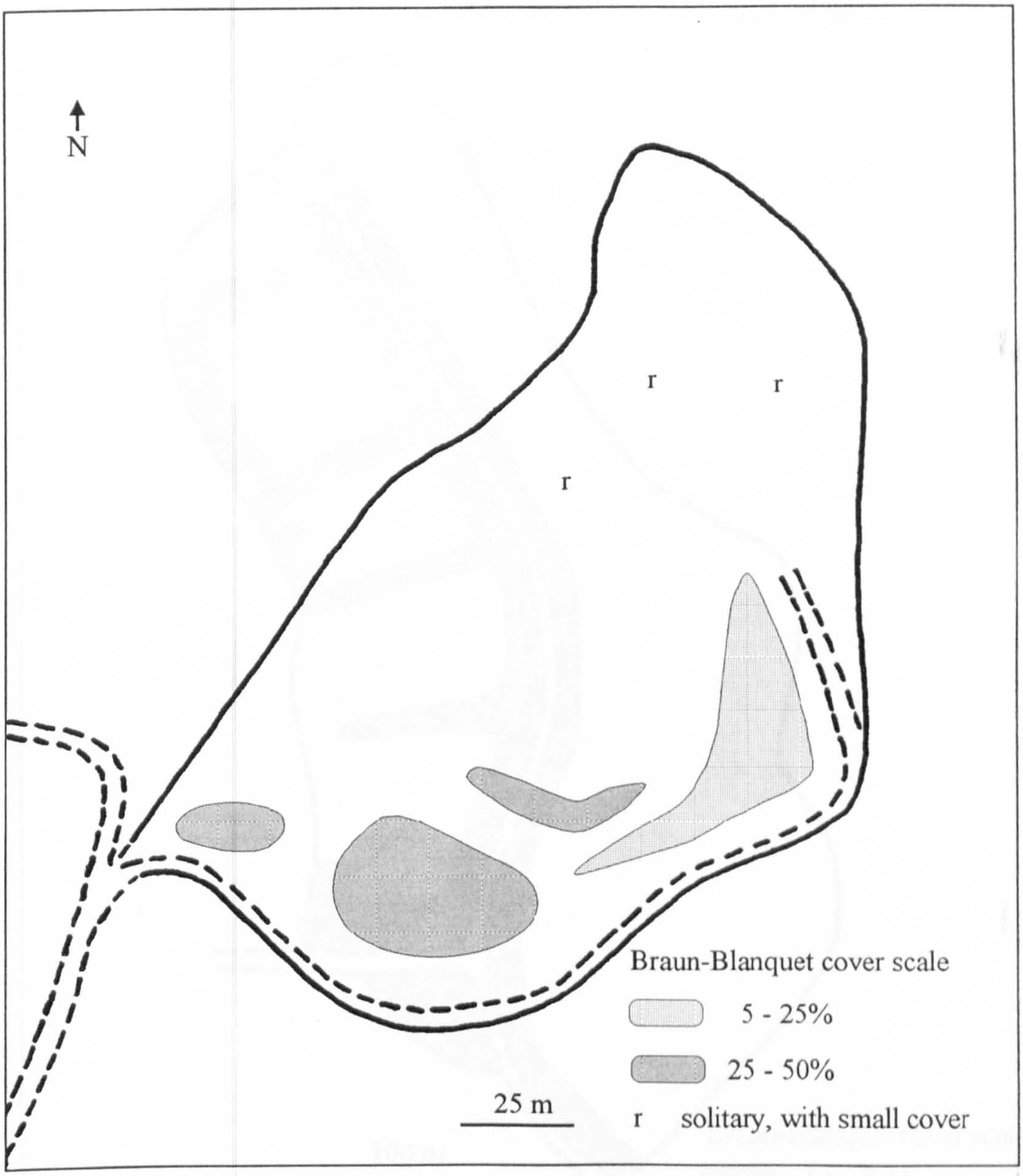


Fig. 3.1 Schematic diagram of tree cover at Ngau Tam Mei Landfill.

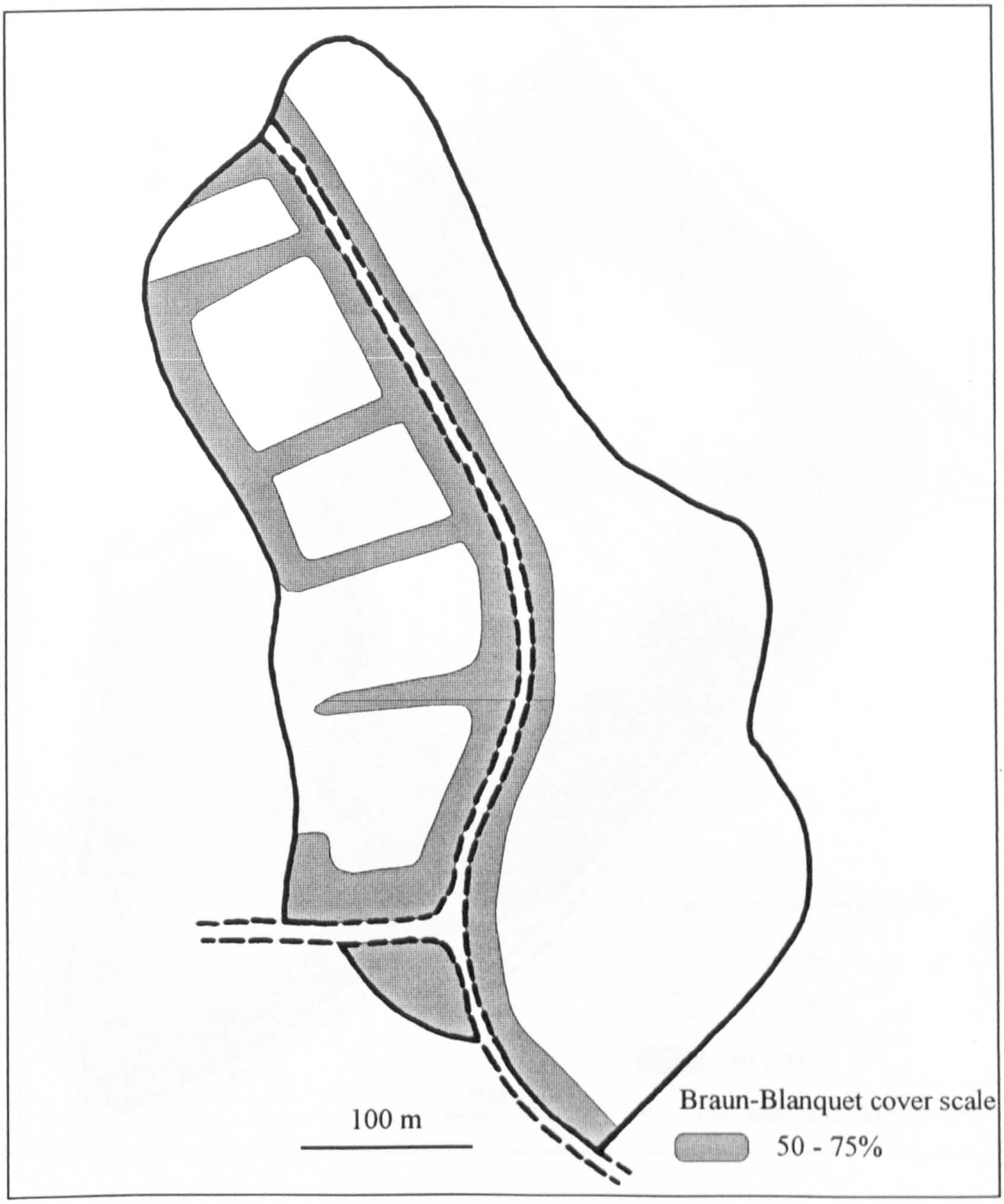


Fig. 3.2 Schematic diagram of Ngau Chi Wan Landfill.

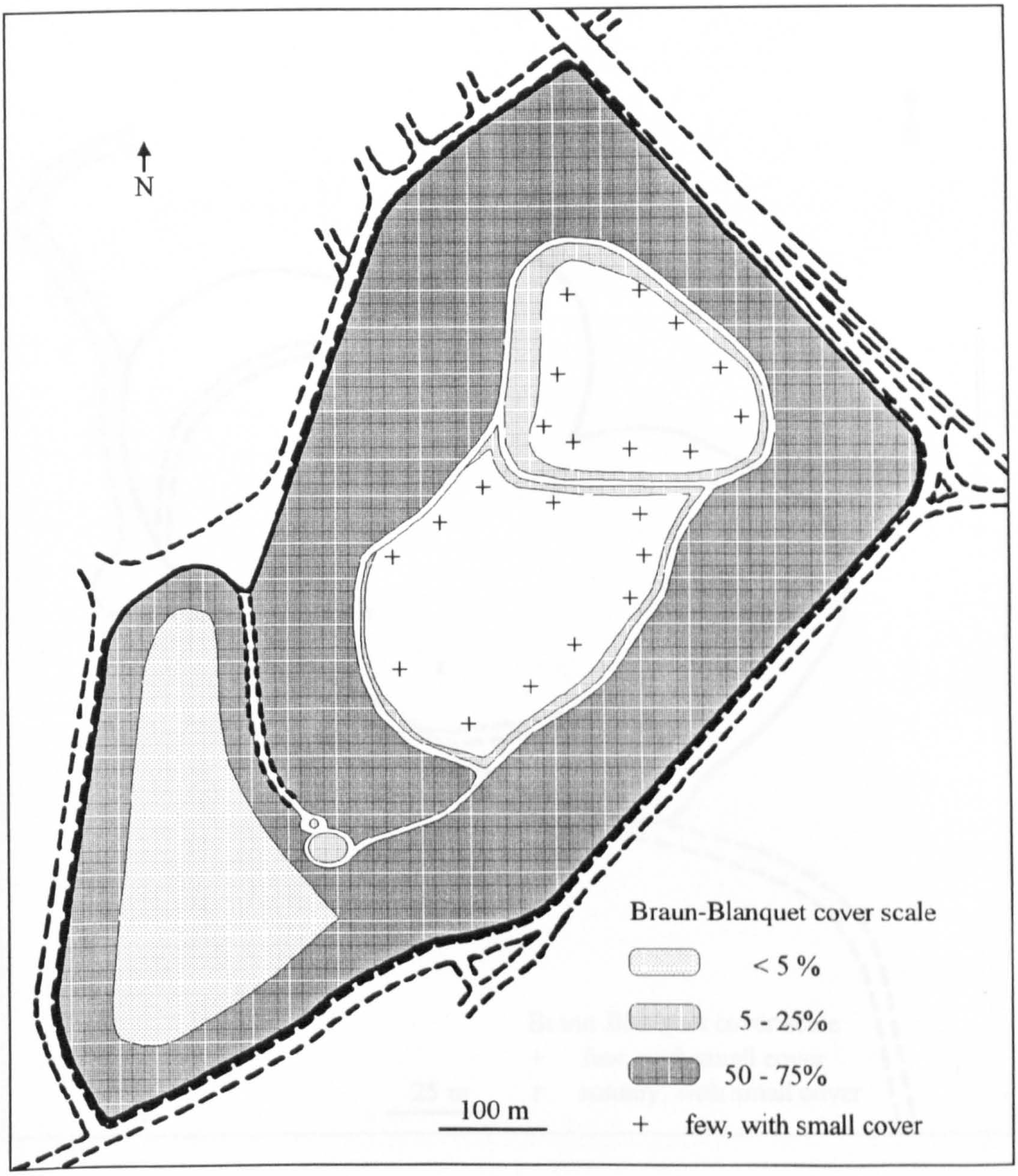


Fig. 3.3 Schematic diagram of Gin Drinkers' Bay Landfill.

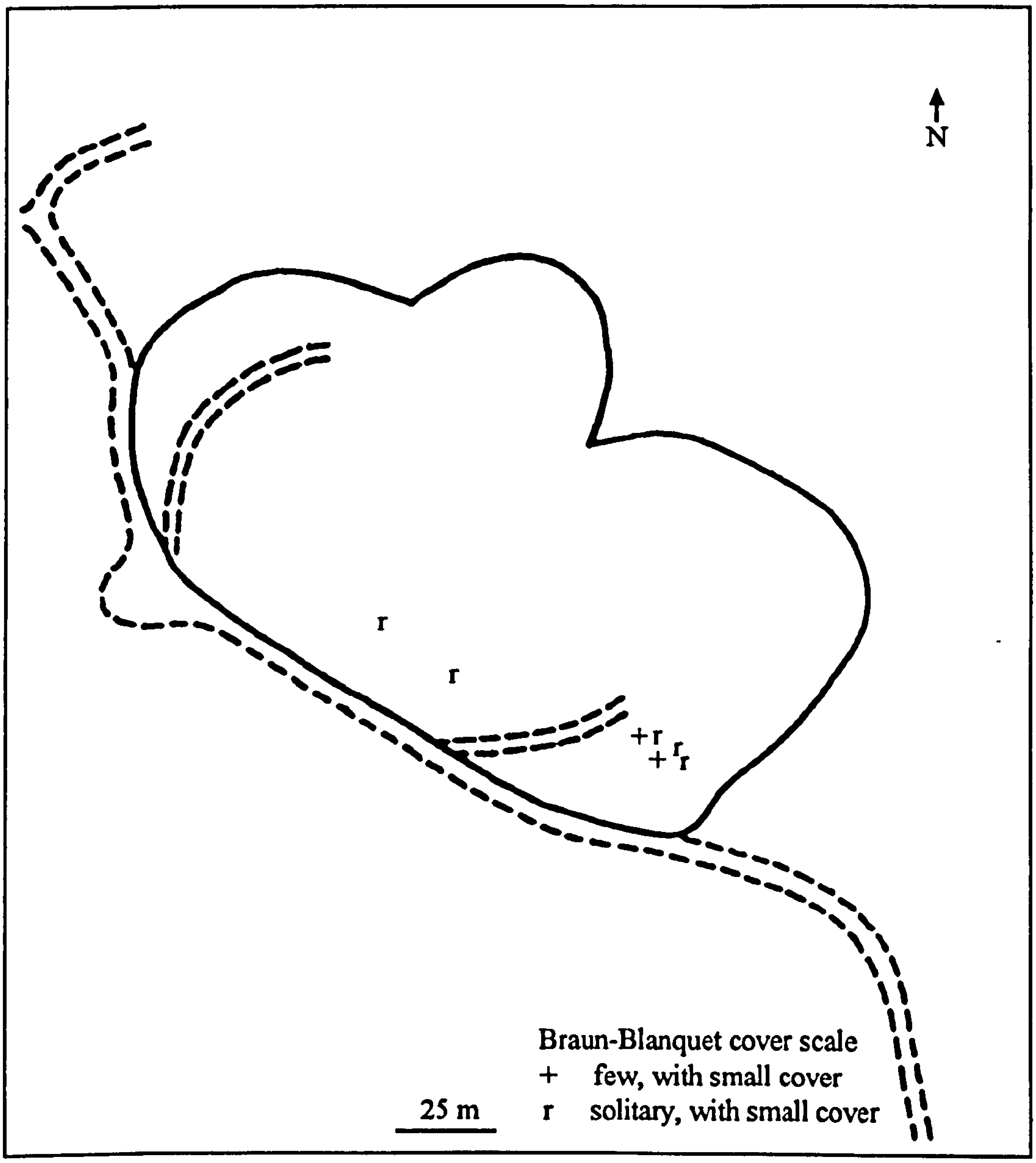


Fig. 3.4 Schematic diagram of Ma Tso Lung Landfill.

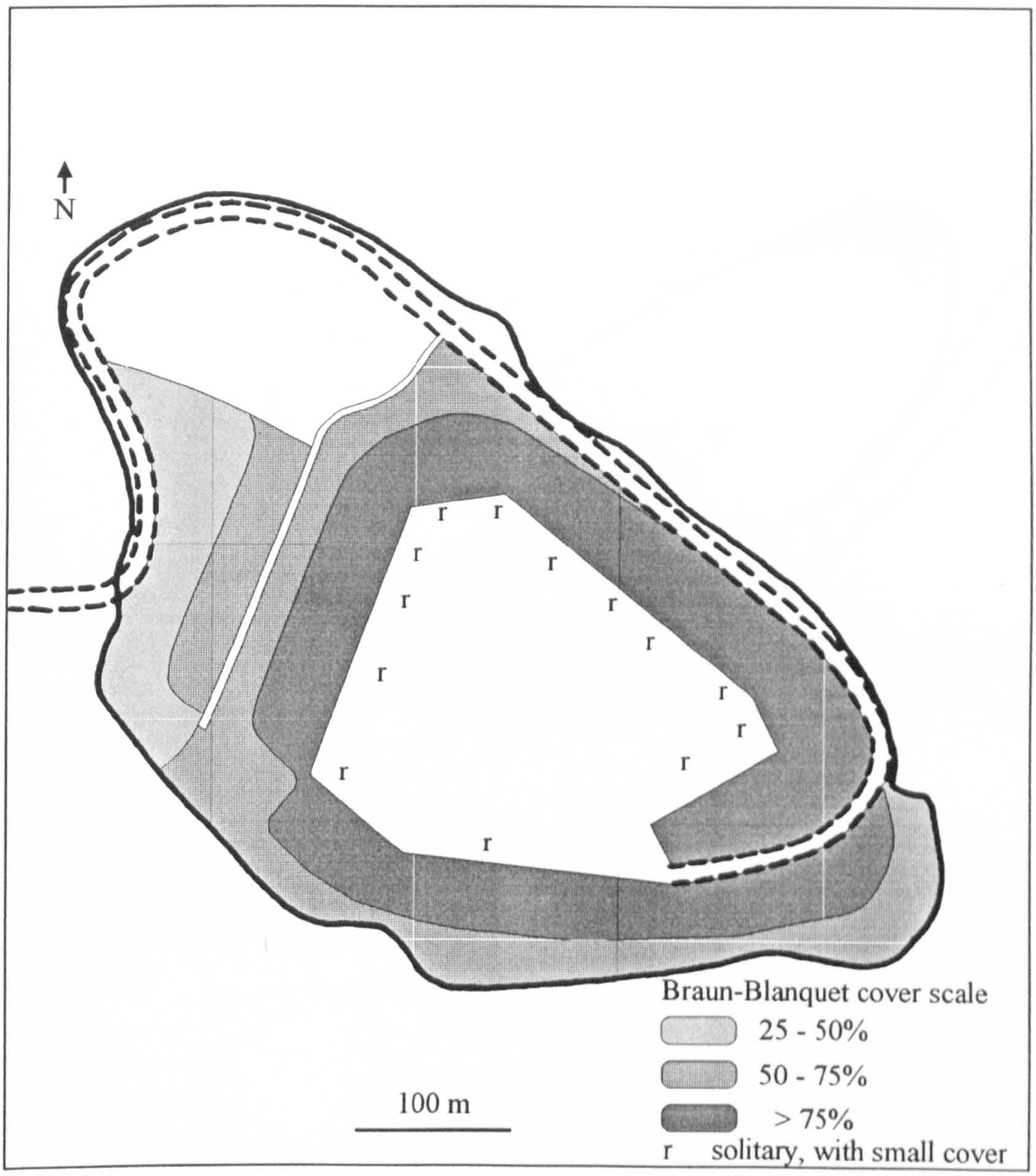


Fig. 3.5 Schematic diagram of Sai Tso Wan Landfill.

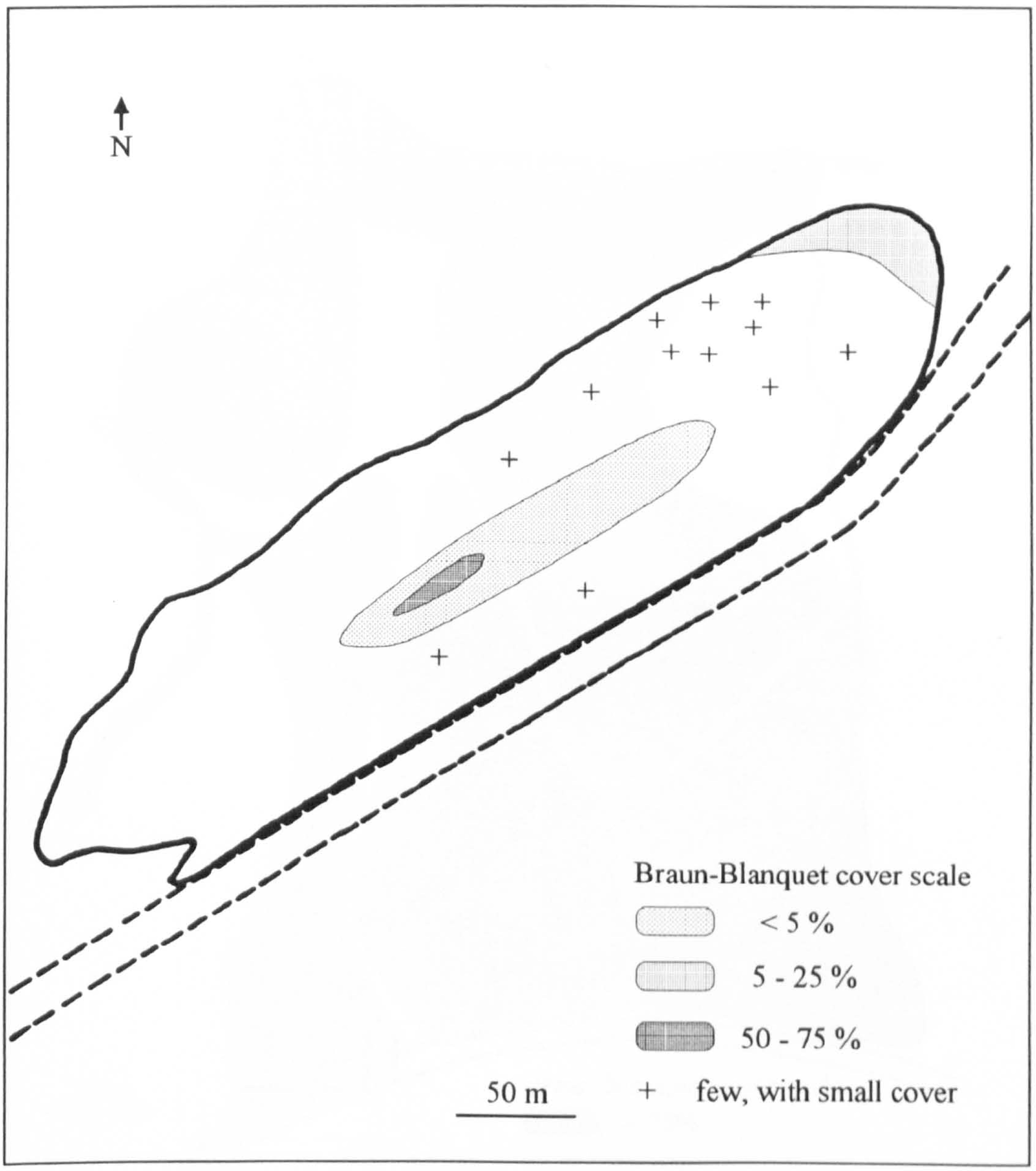


Fig. 3.6 Schematic diagram of Ma Yau Tong West Landfill.

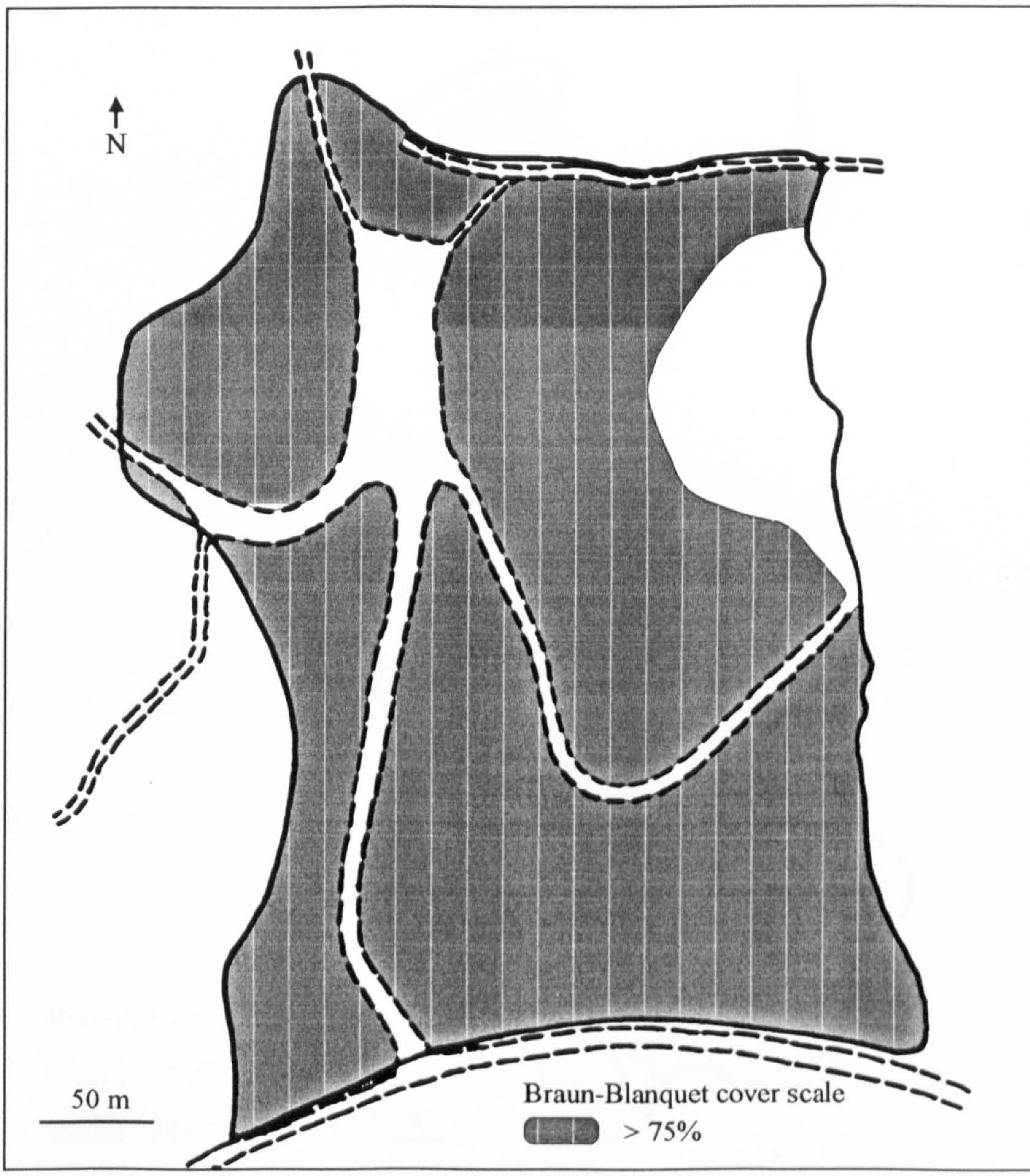


Fig. 3.7 Schematic diagram of Siu Lang Shui Landfill.

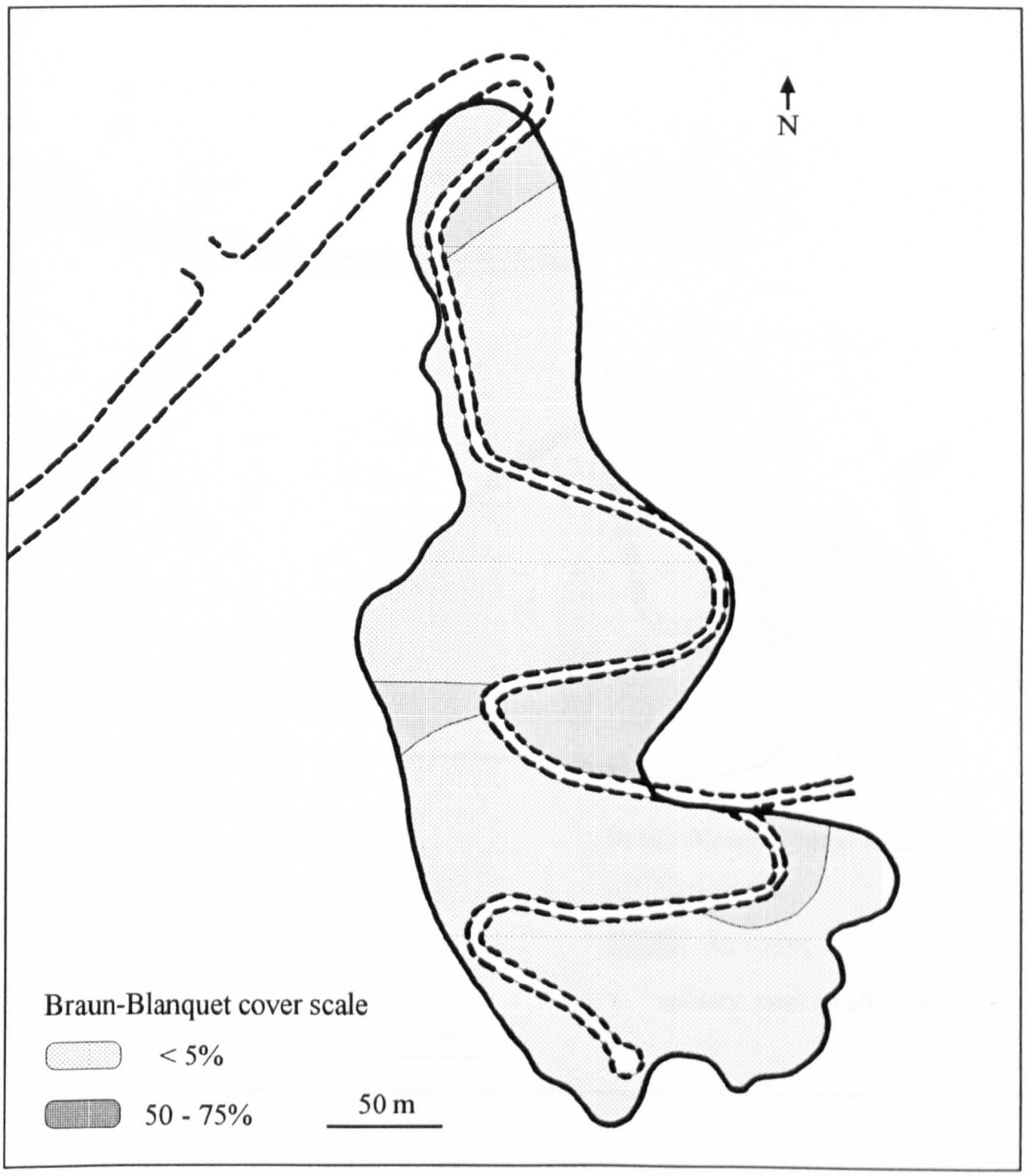


Fig. 3.8 Schematic diagram of Ma Yau Tong Central Landfill.

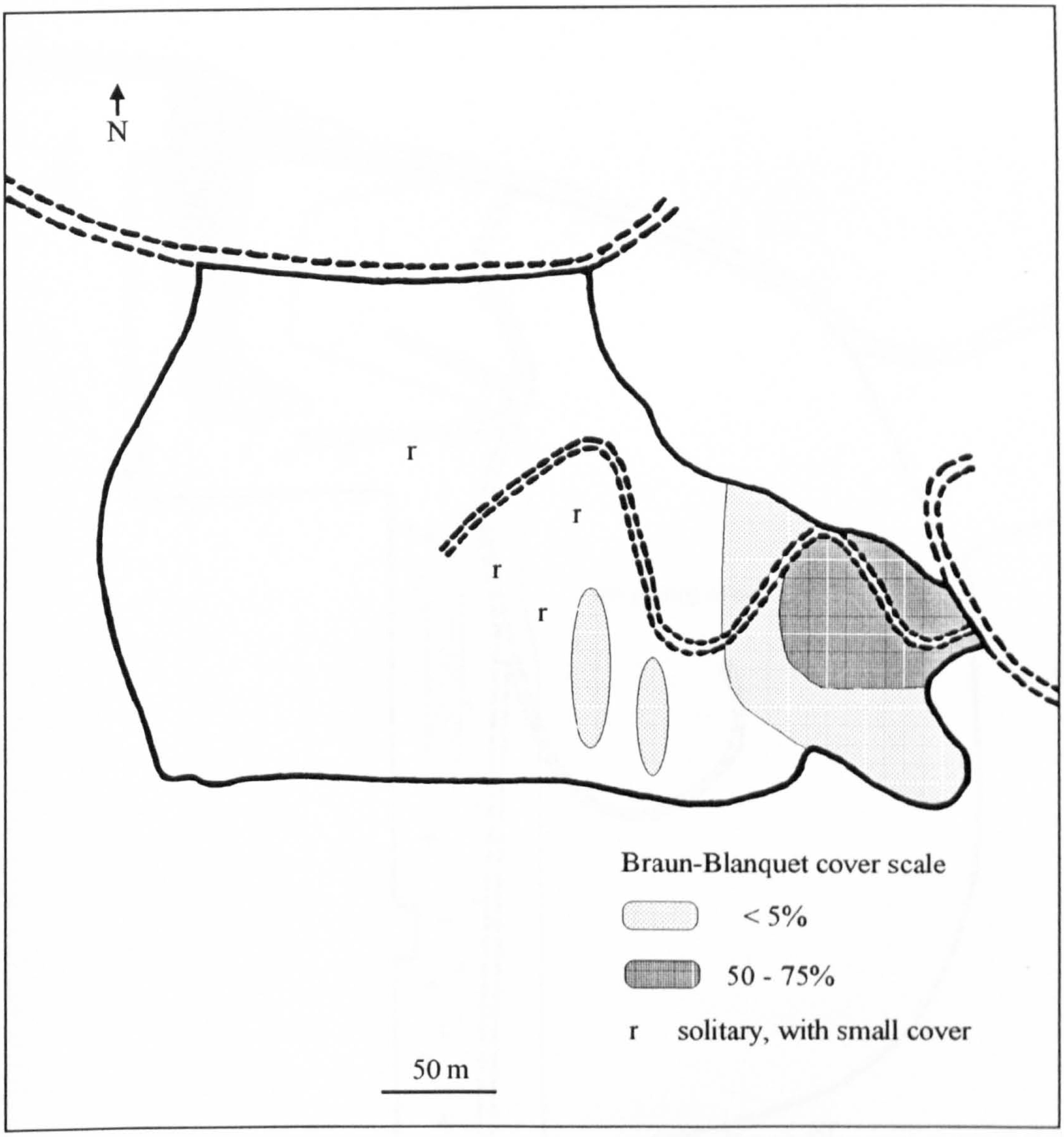


Fig. 3.9 Schematic diagram of Jordan Valley Landfill.

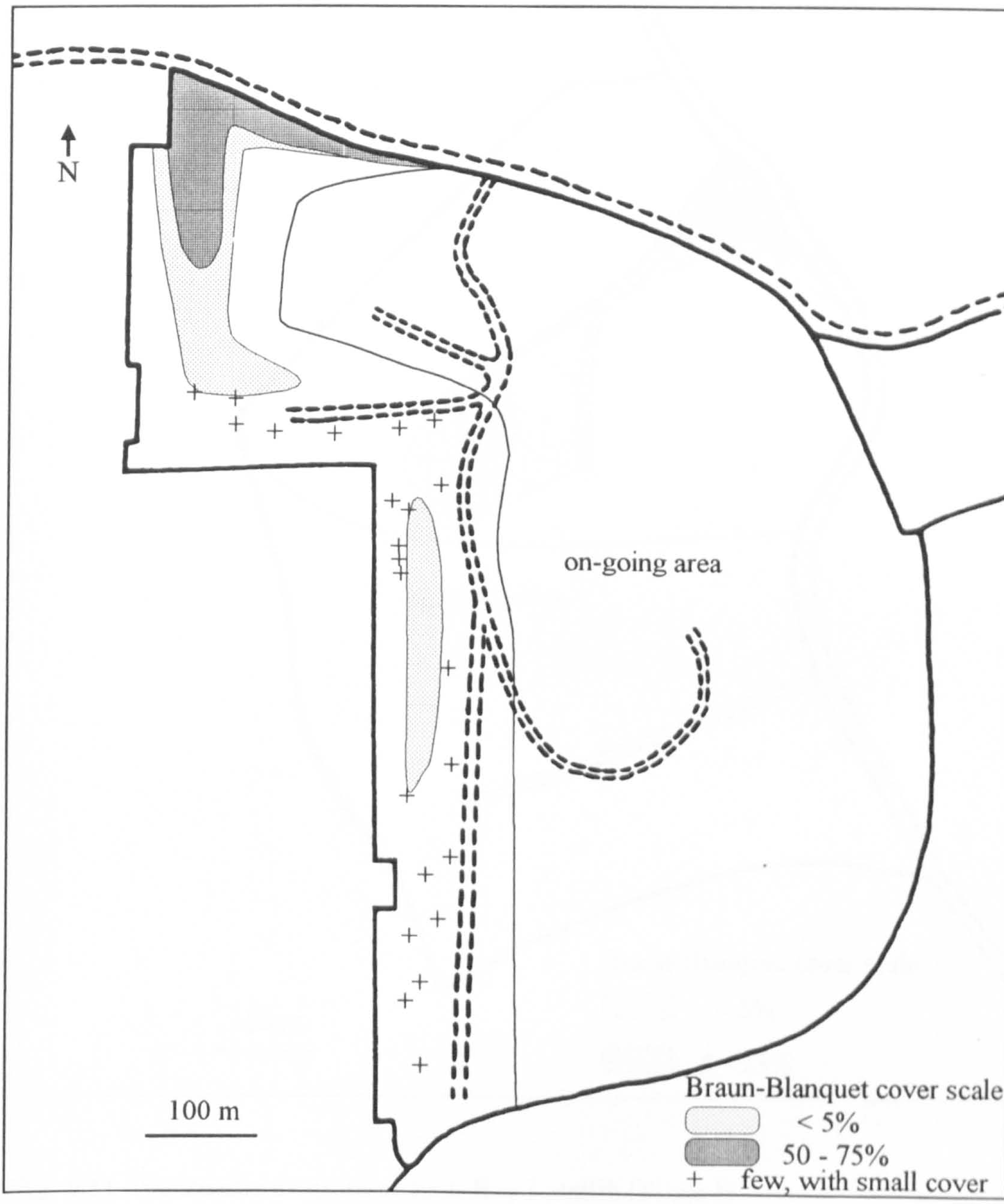


Fig. 3.10 Schematic diagram of Shuen Wan Landfill.

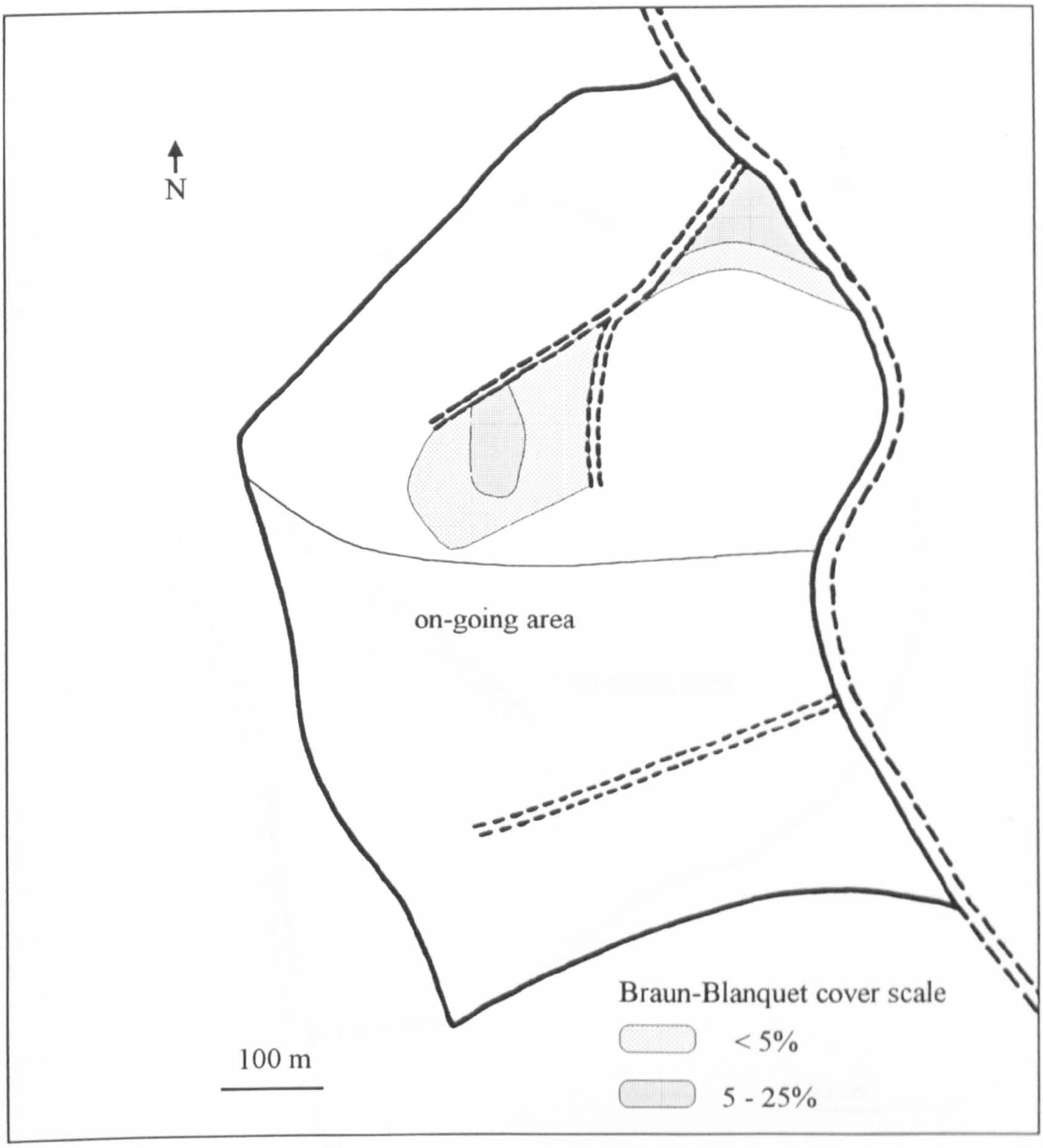


Fig. 3.11 Schematic diagram of Junk Bay Landfill (Stage I).

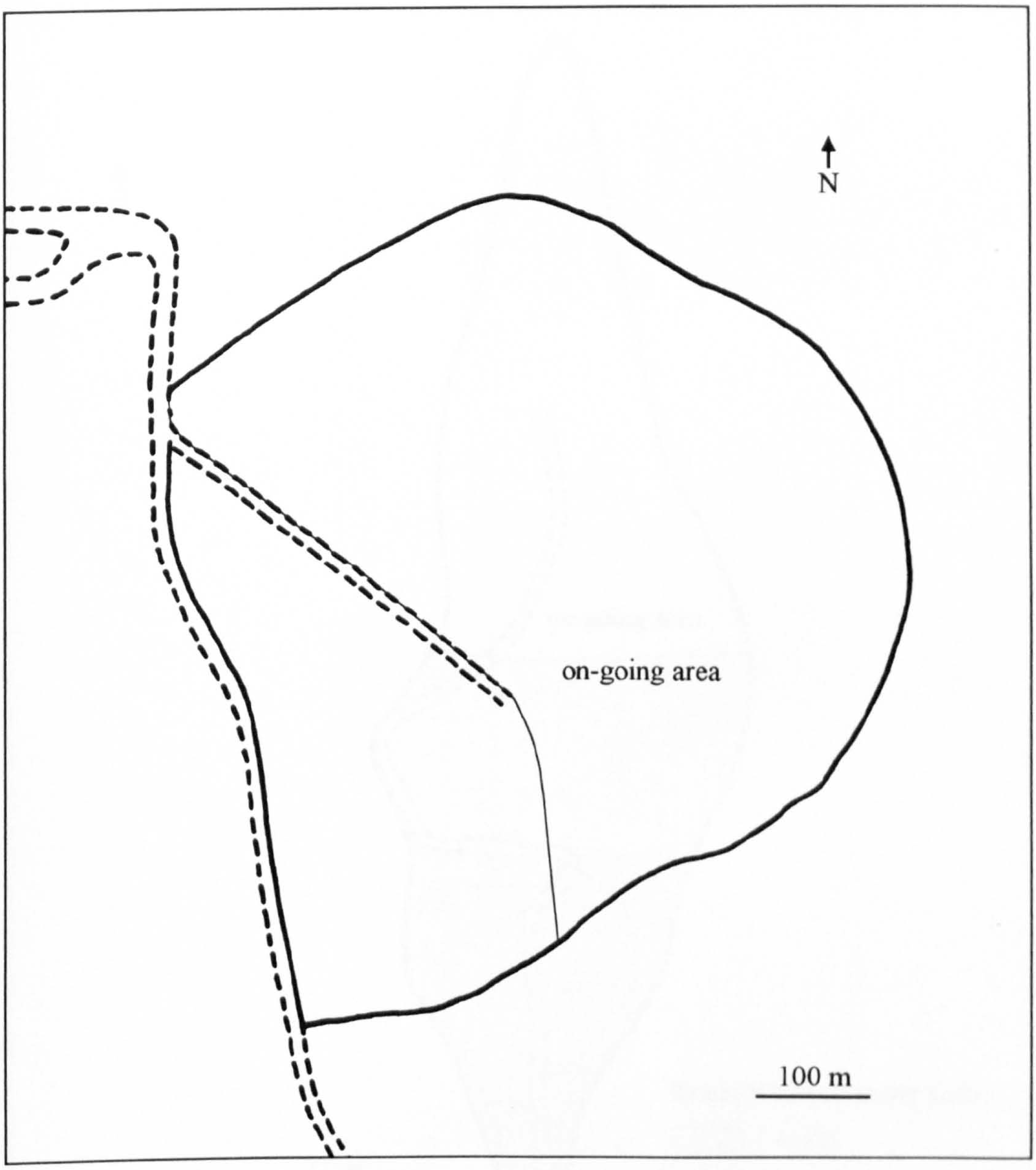


Fig. 3.12 Schematic diagram of Junk Bay Landfill (Stage II/III).

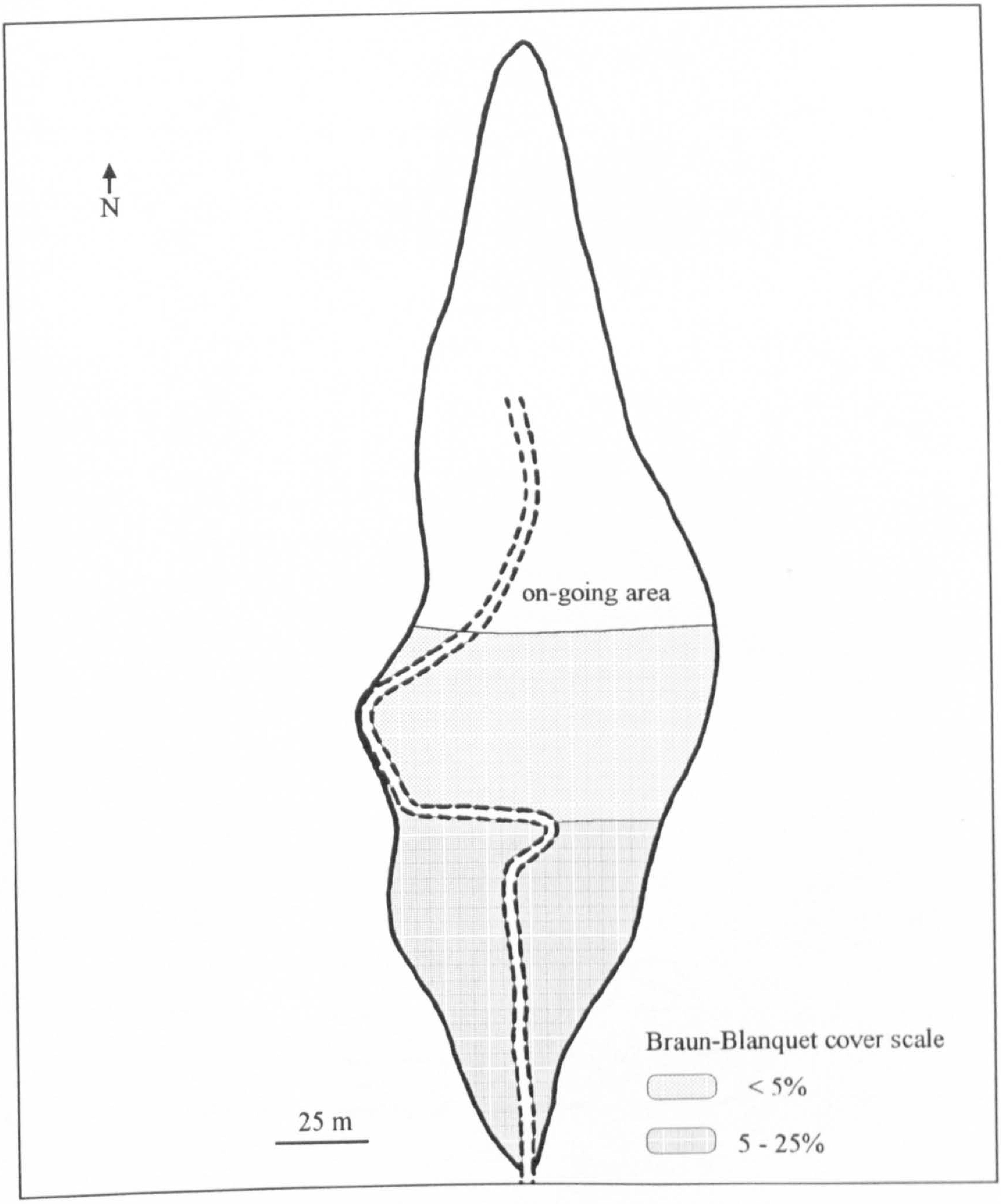


Fig. 3.13 Schematic diagram of Pillar Point Landfill.

3.5 Relationship between soil conditions and tree cover

An analysis was made of the relationship between tree cover and soil conditions at each site (Table 3.5) Gaseous concentrations (N_2, CH_4, O_2 and CO_2) were highly correlated with each other ($|r| > 0.877$). The coefficients of correlation among tree cover and gaseous concentrations ($|r| > 0.381$) was higher than the coefficients of correlation among tree cover and other soil conditions ($|r| < 0.343$).

The relative tree covers of legumes and non-legumes on landfill sites were analyzed (Tables 3.6, 3.7). Within the total 268.1 ha of the 13 sites, 136.1 ha (50.8%) were roads, vehicle parking space and active dumping areas. The remaining 132.0 ha (49.2%) were landscaped or covered with plants. In total, only 34.1 ha (25.8%) of landscaped land was covered by trees. The remaining 74.2% was bare. Forty-one species were found on the sites: 10 were legumes and 31 were non-legumes. Within the 34.1 ha of tree-covered land, 22.3 ha was covered by legumes and 11.8 ha by non-legumes. When the relative cover of legumes and non-legumes was compared, the former higher (65.4%) than the latter (34.6%).

Acacia confusa was the most abundant leguminous tree. 55.1% of the total landfill land which had a tree cover was covered by this species. Presumably, most A. confusa were transplanted to sites by landscape contractors. However, self-seeded young seedlings were also found commonly on most sites. They had uneven height and random occurrence.

Leucaena leucocephala was the second most abundant legume, its overall relative cover was 5.3%. Evidence that it had not been transplanted to the sites was provided by its random occurrence and uneven height.

Table 3.5 Coefficient of correlations between soil conditions and tree cover at the 13 landfill sites. Refer to Tables 3.1 and 3.2 for the data of soil conditions. The percentage of tree cover does not include cemented or on-going areas (see Table 3.6); $P = 0.05$.

	CO_2	CH_4	O_2	N_2	bulk density	moisture	NH_3	NO_2	NO_3	tree cover
CO_2	1.000									
CH_4	0.965	1.000								
O_2	-0.975	-0.901	1.000							
N_2	-0.949	-0.979	0.877	1.000						
bulk density	-0.170	-0.170	0.125	0.163	1.000					
moisture	0.494	0.499	-0.395	-0.531	-0.665	1.000				
NH_3	-0.031	-0.021	0.011	0.009	0.776	-0.485	1.000			
NO_2	-0.058	-0.017	0.096	0.083	-0.462	0.497	-0.275	1.000		
NO_3	0.101	0.165	-0.015	-0.106	0.193	0.175	0.255	0.107	1.000	
tree cover	-0.415	-0.409	0.440	0.381	-0.343	-0.181	-0.031	0.158	-0.215	1.000

Table 3.6 Relative tree cover, number of species and total number of trees at the 13 landfill sites. u = on-going site.

	Ngau Tam Mei	Ngau Chi Wan	Gin Drinkers' Bay	Ma Tso Lung	Sai Tso Wan	Ma Yau Tong West	Siu Lang Shui	Ma Yau Tong Central	Jordan Valley	Shuen Wan[u]	Junk Bay Stage I[u]	Junk Bay Stage II/III[u]	Pillar Point Valley[u]
site area (ha)	2.0	13.5	29.0	2.0	14.0	6.6	11.7	5.8	6.5	48.0	60.0	35.0	34.0
landscaped land													
area (ha)	1.9	4.1	28.0	1.9	13.3	6.3	10.5	5.5	6.2	9.6	24.0	8.8	11.9
relative area of site (%)	95	30	95	95	95	95	90	95	95	20	40	25	35
tree cover													
area (ha)	0.019	2.46	11.2	0.002	5.99	0.315	9.45	0.55	0.31	0.19	2.40	0	1.19
relative cover on landscaped land (%)	1	60	40	0.1	45	5	90	10	5	2	10	0	10
No. of species	5	12	20	1	6	7	7	11	8	7	7	0	5
No. of tree	32	53	3060	2	1112	205	18500	309	78	104	548	0	90

Table 3.7 Summary of overall tree cover at the 13 landfill sites.

tree cover on landscaped land		area (ha)		relative cover (%)
legumes (10 spp.)				
Acacia confusa		18.8		55.1
Leucaena leucocephala		1.8		5.3
others 8 spp.		1.7		5.0
		22.3		65.4
non-legume (31 spp.)		11.8		34.6
		34.1	total	100.0
bare ground		97.9		
		132.0		
cemented or active pumping area		136.1		
	total	268.1		

CHAPTER 4
EFFECTS OF LANDFILL GAS

4.1 Overview

The field survey indicated that legumes were relatively abundant at most landfill sites, which often have high concentrations of CO_2 and CH_4 and low concentrations of O_2 at the landfill cap (Chapter 3). Although Rhizobium-legume N_2 fixation under sub-ambient concentrations of O_2 has been described in some legume herbs and crops (1.311), similar information for woody legumes appears to be lacking. Furthermore, similar information on N_2 fixation at high concentrations of CO_2 and CH_4, as would happen on landfill soil, is not available (1.312). To investigate the influence of landfill gas on symbiotic N_2 fixation, laboratory studies were planned for the two most abundant legume trees on landfill sites shown in the field survey: Acacia confusa and Leucaena leucocephala. The studies consisted of short-term tests (1-h) and long-term tests (4-week).

4.2 Short-term assay

4.21 Preparation of assay system

Acacia confusa and Leucaena leucocephala seedlings were prepared as described in Section 2.5. Culture nutrient solution free of combined N (2.54) was supplied to the young seedlings. Rhizobial infection in seedlings was established by artificial inoculation at least two months before the assay (2.412).

For each species of legume, the C_2H_2 reduction of nodulated roots at serial concentrations of O_2, CO_2 and CH_4 (Table 4.1) was studied at 15-min intervals for 1 h. The preparation of the serial dilution of O_2 is described in Section 2.7. The experimental procedures to study the influence of CO_2 and CH_4 were similar to the procedure applied in the O_2 assay. However, in the presence of high concentrations of CO_2, the O_2 on landfill topsoil would be depleted from the ambient concentration (20.95%). Therefore, to study the C_2H_2 reduction of nodules under different concentrations of CO_2 and CH_4, the O_2 concentration was fixed at a sub-ambient concentration of 16.0%.

Table 4.1 Gaseous composition of the 35-ml bottles used to assay the influence of O_2, CO_2 and CH_4 concentrations on the ARA of root nodules.

assay on O_2

	O_2 (% v v^{-1})					
	10.0	20.0	40.0	80.0	16.0	20.0
gas	volume (ml)					
O_2	0.37	0.73	1.46	2.93	5.85	7.31
C_2H_2	1.75	1.75	1.75	1.75	1.75	1.75
Ar	32.89	32.52	31.79	30.32	27.40	25.94
total	35.00	35.00	35.00	35.00	35.00	35.00

assay on CO_2 or CH_4

	CO_2 or CH_4 (% v v^{-1})					
	0	10.0	20.0	30.0	40.0	50.0
gas	volume (ml)					
CO_2 or CH_4	0	3.50	7.00	10.50	14.00	17.50
O_2	5.85	5.85	5.85	5.85	5.85	5.85
C_2H_2	1.75	1.75	1.75	1.75	1.75	1.75
Ar	32.88	29.38	25.88	22.38	18.88	15.38
total	35.00	35.00	35.00	35.00	35.00	35.00

4.22 Short-term effect of landfill gas

A preliminary time-course study on C_2H_2 reduction of Leucaena leucocephala nodule was conducted to find out the best assay period and the general trend of response to different concentrations of O_2 and CO_2. The results indicated a broadly linear C_2H_2 reduction within 1 h at high O_2 and low CO_2 conditions and a decline in C_2H_2 reduction was found within 1 h at low O_2 and high CO_2 conditions (Table 4.2). Similar C_2H_2 reduction test which lasted for 2 h (results not shown) indicated the C_2H_2 reduction at high O_2 and low CO_2 started to decline soon after 1 h. A 1-h assay was chosen for all subsequent work. In the preliminary test, a maximum 1-h ARA was found at 20.9% O_2 and the ARA decreased as the O_2 concentration decreased in the range of 16.7 - 1%. ARA was depressed in the presence of 10 - 50% CO_2; minimum ARA at 50% CO_2 was comparable to the ARA at 4.2 - 8.4% O_2.

The short-term assay on Leucaena leucocephala was repeated when more mature and infected seedlings and apparatus for gas transfer and gas dilution were available. This time, the tests included Acacia confusa and CH_4. The concentrations of O_2, CO_2 and CH_4 were set to cover the common concentrations of these gases in landfill topsoil: O_2) 20 - 1%, CO_2) 10 - 50% and CH_4) 10 -50%. As the ARA of nodules depends highly on the physiological status of the seedlings (1.3), it is difficult to compare the values of ARA of nodules from different batches of legume host. Therefore, only the results of the second test were described in detail (Fig. 4.1 - 4.5) and comparison was made on 1-h ARA at different concentrations of O_2, CO_2 and CH_4 and on the two legumes.

Table 4.2 Short-term influence of O_2 and CO_2 concentrations in C_2H_2 reduction (μmol C_2H_4 g^{-1} f. wt) on Leucaena leucocephala nodules. C_2H_2 reduction of nodulated roots was determined at 15 min interval for 1 h at room temperature (21°C). O_2 concentration was 16.0% in the CO_2 assay.

O_2

	ARA							
time	15 min		30 min		45 min		60 min	
	mean	SD	mean	SD	mean	SD	mean	SD
O_2 (% v v^{-1})								
20.9	1.58	0.300	3.36	0.87	5.15	1.23	7.00	1.63
16.7	0.879	0.356	1.73	0.759	2.51	1.10	3.74.	1.02
8.4	0.304	0.063	0.630	0.178	1.09	0.24	1.69	0.22
4.2	0.158	0.046	0.243	0.110	0.282	0.173	0.559	0.313
2.1	0.108	0.029	0.108	0.032	0.111	0.033	0.120	0.059
1.0	0.0902	0.0255	0.0887	0.0231	0.0924	0.0230	0.117	0.035

CO_2

	15 min		30 min		45 min		60 min	
	mean	SD	mean	SD	mean	SD	mean	SD
CO_2 (% v v^{-1})								
10	0.822	0.057	1.46	0.30	2.20	0.44	2.91	0.63
20	0.529	0.266	0.965	0.467	1.28	0.59	1.55	0.69
30	0.687	0.175	1.07	0.34	1.23	0.35	1.28	0.39
40	0.313	0.172	0.540	0.339	0.658	0.414	0.755	0.447
50	0.330	0.174	0.530	0.351	0.606	0.663	0.652	0.459

The influence of O_2 concentration on nodular ARA in both legumes was similar (Fig. 4.1). Maximum ARA was found at 20.0% O_2 and the ARA decreased as the O_2 decreased in the range of 16 - 1%. Within the 1-h study period, the nodular C_2H_2 reduction at 8 and 16% O_2 in both legumes increased slightly after the first 30-min. The 1-h ARA of Acacia confusa nodule at 20 and 16% O_2 was significantly higher than the ARA at $\leq$ 8% O_2 ($P < 0.05$) (Fig 4.4). However, the 1-h ARA of Leucaena leucocephala nodules at $\leq$ 16% was significantly lower than the ARA at 20% O_2 (Fig. 4.5). The 1-h ARA at 16% was 9% less than the ARA at 8% O_2 ($P > 0.05$). For both legumes, extremely low level of 1-h ARA at $\leq$ 4% O_2 was determined and it was significantly lower than the ARA at $\geq$ 8% O_2 ($P < 0.05$).

The influence of CO_2 on nodular ARA was tested at concentrations commonly found in landfill topsoil (10 - 50%) (Fig. 4.2). Generally, high CO_2 caused a depression in the ARA of Acacia confusa nodules. At 30 - 50% CO_2, the 1-h ARA was mainly caused by the C_2H_2 reduction within the first 30 min. The C_2H_2 reduction at 10 - 20% CO_2 was relatively stable throughout the 1-h period. The 1-h ARA at 30 - 50% CO_2 was significantly lower than the 1-h ARA at 0 - 20% CO_2 ($P < 0.05$) (Fig. 4.4).

The 1-h ARA of Leucaena leucocephala nodules at different CO_2 concentrations was different from the response of Acacia confusa nodules to CO_2. The 1-h ARA of Leucaena leucocephala nodules was markedly induced by 10% CO_2 ($P < 0.05$). The amount of C_2H_4 formed within the 30 - 60 min by nodules at 20 - 30% CO_2 was similar to those nodules at 0% CO_2. The higher 1-h ARA of 20 - 40% CO_2 in comparison to 0% CO_2 was mainly due to the C_2H_2 reduction of the nodules within the first 15 - 30 min. Moreover, the 1-h ARA at 20 - 40% CO_2 was not significantly higher than the 1-h ARA at 0% CO_2 ($P > 0.05$) (Fig. 4.5). C_2H_2 reduction of nodules at 50% CO_2 was hardly detectable after the first 15 min.

No special pattern or trend was observed in C_2H_2 reduction of either legume at 0 - 50% CH_4 within the 1-h assay period and there was no significant difference ($P > 0.05$) in the 1-h ARA of nodules of either legume over the same range of CH_4. The variation in 1-h ARA (e.g. low ARA of Acacia confusa at 10% CH_4 and high ARA of Leucaena leucocephala at 10% CH_4) was very likely due to the unavoidable experimental artefact which is to be explained in Chapter 7.

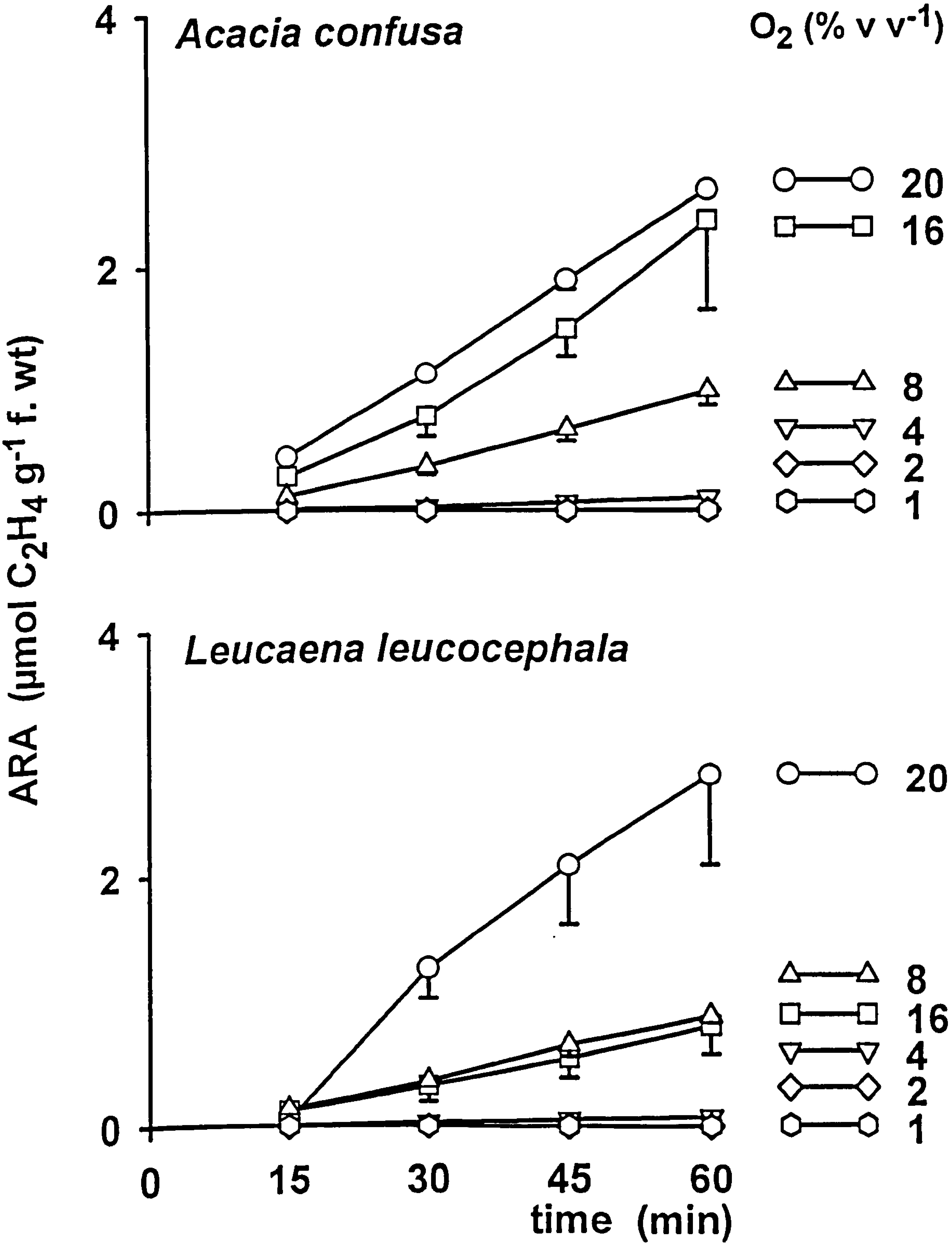


Fig. 4.1 Influence of O_2 on C_2H_2 reduction of nodules of two legumes measured at 15-min intervals for 1 h. Exact gaseous composition listed in Table 4.1; assayed at room temperature (Sections 2.7, 4.21).

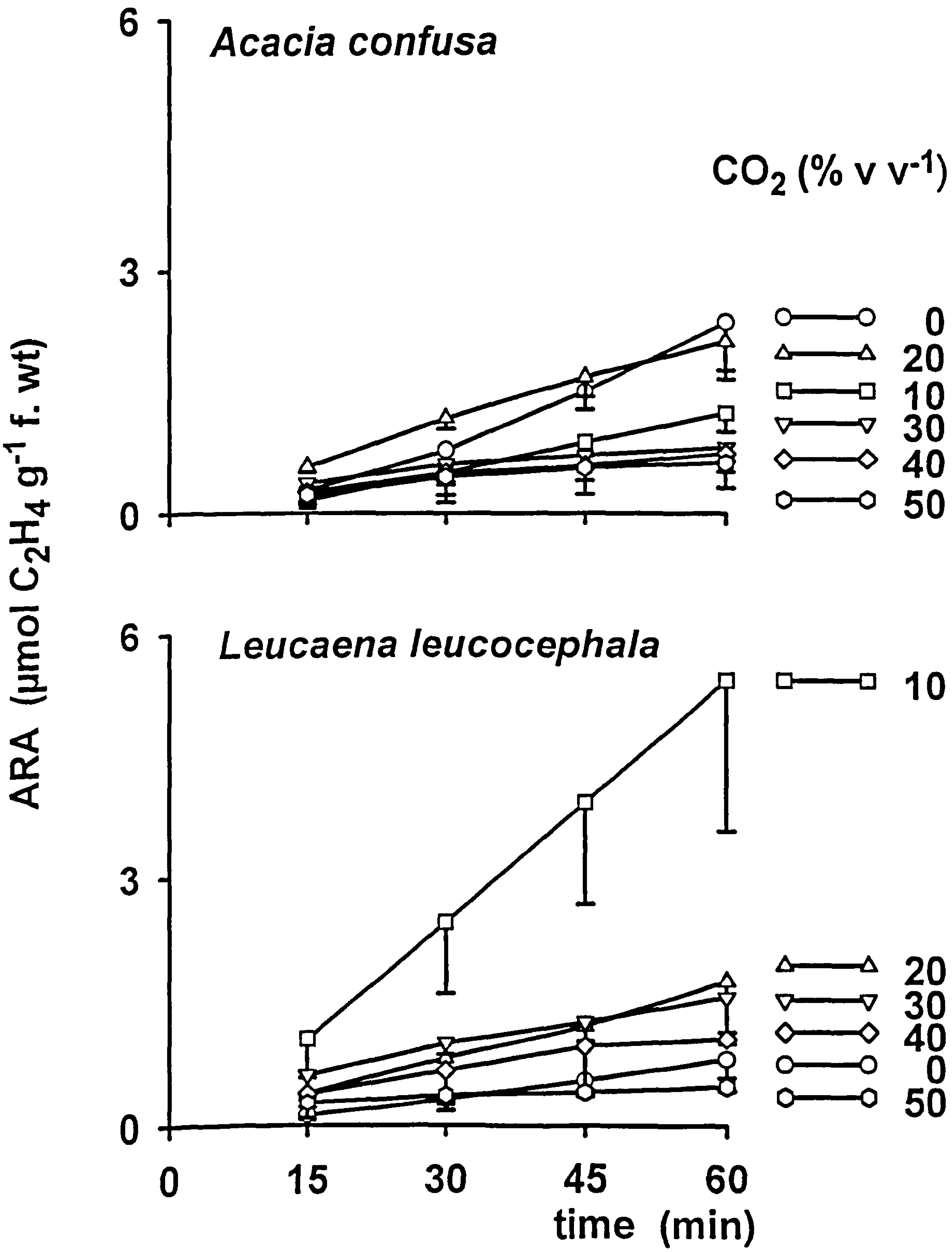


Fig. 4.2 Influence of CO_2 on C_2H_2 reduction of nodules of two legumes measured at 15-min intervals for 1 h. Exact gaseous composition listed in Table 4.1; assayed at room temperature (Sections 2.7, 4.21).

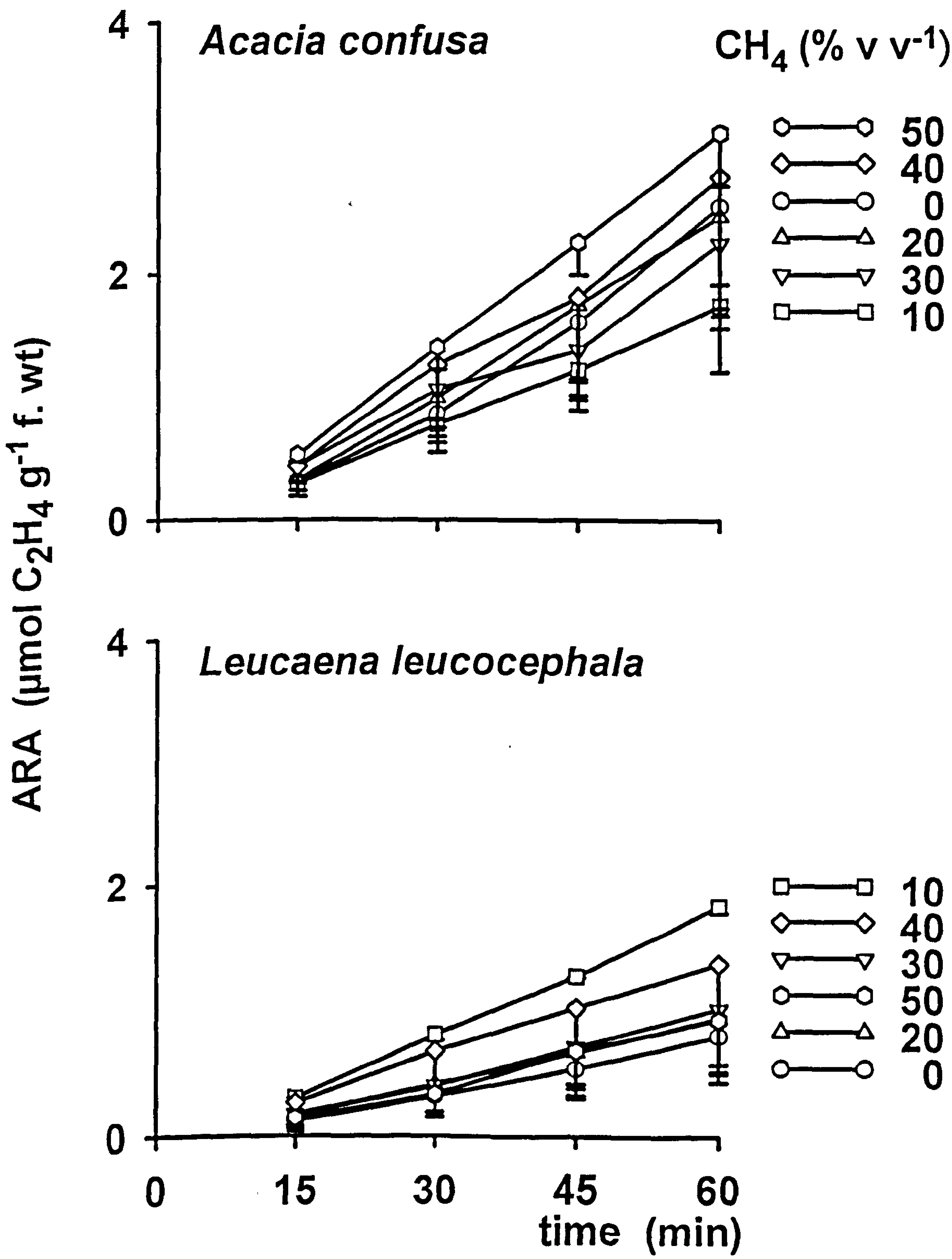


Fig. 4.3 Influence of CH_4 on C_2H_2 reduction of nodules of two legumes measured at 15-min intervals for 1 h. Exact gaseous composition listed in Table 4.1; assayed at room temperature (Sections 2.7, 4.21).

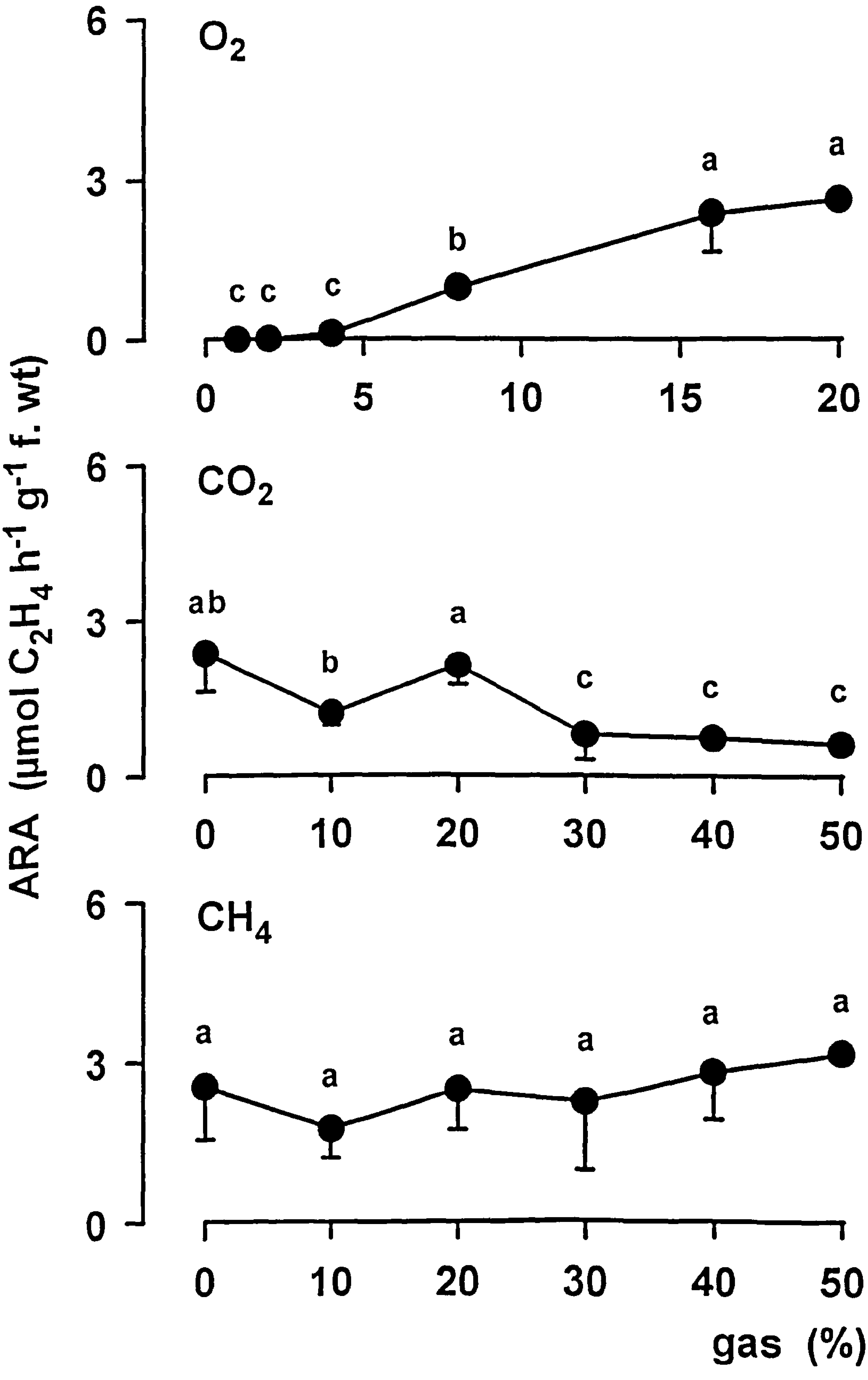


Fig. 4.4 Influence of O_2,CO_2 and CH_4 on 1-h ARA of Acacia confusa nodules. Exact gaseous composition listed in Table 4.1; assayed at room temperature. Same symbol on a graph indicates no significant difference ($P < 0.05$).

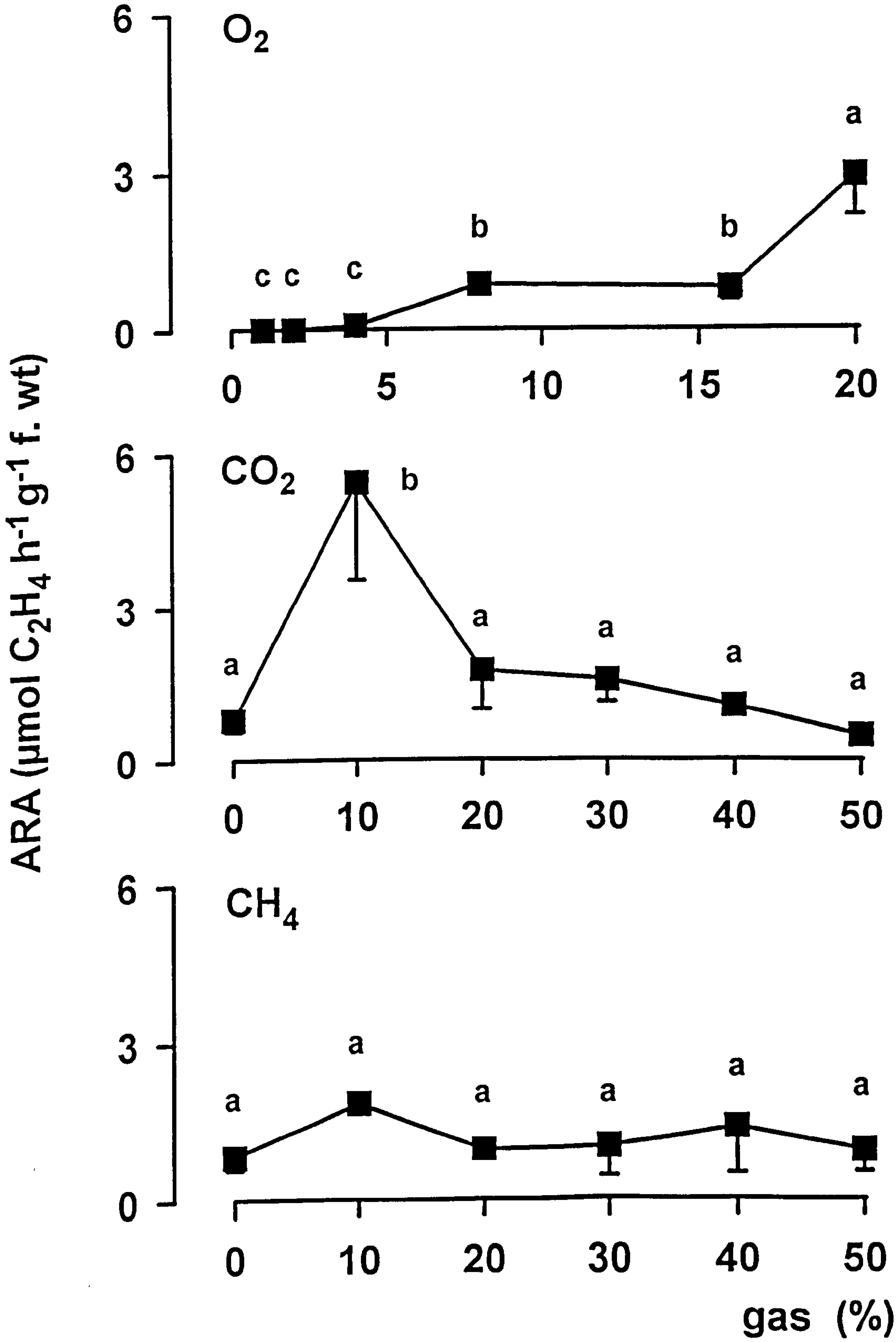


Fig. 4.5 Influence of O_2 CO_2 and CH_4 on 1-h ARA of Leucaena leucocephala nodules. Exact gaseous composition listed in Table 4.1; assayed at room temperature. Same symbol on a graph indicates no significant difference ($P < 0.05$).

4.3 Long-term assay

4.31 Preparation of the assay system

The long-term influence of landfill gas was studied on the same two species described in Section 4.2. Seedlings were germinated and planted in fumigation chambers (2.54). About 5 ml of N-free nutrient solution (Table 2.5) was added to each bottle about once a week, balanced with sterile distilled water. For each treatment, there were eighteen rhizobia-free seedlings in six fumigation chambers and another eighteen rhizobia-infected seedlings in another six chambers. The preparation of rhizobia-infected seedlings was described as in Section 2.412.

The influence of landfill gas and CO_2 was studied on three-month old Leucaena leucocephala seedlings. Only the influence of landfill gas was studied on Acacia confusa seedlings, as the facility for CO_2 fumigation was not available.

Landfill gas and CO_2 to fumigate seedlings were prepared as in Section 2.6. At least one digester was connected to a fumigation chamber and the gas flow was adjusted to about 10 ml min^{-1}.

After four weeks, plant samples were harvested for biomass measurements. The ARA of nodulated roots was tested at 95% ambient air and 5% C_2H_2 (Section 2.7).

4.32 Long-term effect of landfill gas

The long-term influence of landfill gas on rhizobia-legume system was studied and the ARA of nodules after gas treatments was compared. The simulated landfill gas caused a 1.5 times increase in N_2-fixing activity per unit f. wt of Acacia confusa nodules (Table 4.2). However, in terms of C_2H_2 reduction per total nodules of individual plant, landfill gas did not cause any significant increase, as the f. wt of harvested nodules was less than the control. Rhizobia-infected seedlings had a higher mean plant biomass (273 mg) than rhizobia-free seedlings (231 mg). In the control experiment, rhizobia-infected seedlings had 56% higher shoot biomass and 64% higher root biomass than rhizobia-free seedlings.

Landfill gas and CO_2 caused less plant biomass accumulation and lower ARA in Leucaena leucocephala and its associated nodules. The ARA per unit f. wt of nodule (μ mol C_2H_4 h^{-1} g^{-1} f. wt) and the ARA per total nodules of individual plant (μmol C_2H_4 h^{-1} $plant^{-1}$) were significantly reduced by the CO_2 treatment ($P < 0.05$). The landfill gas also caused a 14% reduction in ARA per unit f. wt of nodule and 30% reduction in ARA per nodule of individual plant ($P > 0.05$). Rhizobia-infected seedlings had 26.8 and 8.8% higher biomass under the influence of landfill gas and CO_2, compared with rhizobia-free seedlings.

Landfill gas and CO_2 did not cause any plant death within four weeks; the nodules were effective in fixing N_2 after four months of fumigation. Moreover, under the influence of the gases, rhizobia-infected seedlings had higher biomass than rhizobia-free seedlings.

Table 4.3 Long-term influence of simulated landfill gas and CO_2 on the plant biomass and nodular ARA. One-half of the seedlings was rhizobia-free and the other was infected; assay period was four weeks.

Acacia confusa	control		landfill gas	
	– rhizobia	+ rhizobia	– rhizobia	+ rhizobia
ARA				
(μmol C_2H_4 h^{-1} g^{-1} (f. wt))		1.27 ± 1.82		3.17 ± 1.41
(μmol C_2H_4 h^{-1} $plant^{-1}$)		0.771 ± 0.834		0.767 ± 0.709
seedling biomass				
shoot (mg d. wt)	136 ± 18.7	212 ± 121	158 ± 25.5	206 ± 62.1
root (mg d. wt)	49.6 ± 8.3	81.1 ± 41.5	73.0 ± 12.1	66.5 ± 17.5
nodule biomass (mg f. wt)		42.3 ± 21.7		44.3 ± 16.5

Leucaena leucocephala	control		landfill gas		CO_2	
	– rhizobia	+ rhizobia	– rhizobia	+ rhizobia	– rhizobia	+ rhizobia
ARA						
(μmol C_2H_4 h^{-1} g^{-1} (f. wt))		3.69 ± 1.41		3.17 ± 1.20		0.530 ± 0.158
(μmol C_2H_4 h^{-1} $plant^{-1}$)		50.0 ± 16.6		34.9 ± 18.3		6.10 ± 3.87
seedling biomass						
shoot (mg d. wt)	240 ± 30	314 ± 35	212 ± 22	280 ± 57	225 ± 24	265 ± 39
root (mg d. wt)	118 ± 19	109 ± 18	90.9 ± 10.0	103 ± 13	70.0 ± 4.0	56.0 ± 11.0
nodule biomass (mg f. wt)		42.0 ± 11.0		42.0 ± 9.0		35.0 ± 17.0

CHAPTER 5
EFFECTS OF LANDFILL LEACHATE

5.1 Overview

In addition to landfill gas, contamination of the cover soil by landfill leachate might also threaten trees growing on a completed site (1.12). However, leachate irrigation can be considered as an alternative to leachate treatment and the growth of plants may benefit when the irrigation rate of leachate is adjusted properly (1.122). The presence of high concentrations of NH_4-N is a common characteristic of landfill leachate (Table 1.2) and it is well known that NH_4-N in soil may partially inhibit the growth and activity of legume root nodules (1.332). Therefore, it seems likely that leachate is an effective agent inhibiting N_2 fixation by legume nodules. To verify the influence of leachate on the rhizobia-legume system, the two legumes used in the previous chapter were tested. The regulatory role of the nodules on uptake of combined N from leachate-contaminated-soil was investigated and the responses of legumes and non-legumes were compared.

5.2 Method

Landfill leachate was collected from Shuen Wan Landfill on 12 August 1993, analysed (2.32) and its chemical composition (Table 5.1) was found to be similar to the typical range of landfill leachate (Table 1.2); therefore, it was used to assay the influence of leachate on four species of tree (two legumes, two non-legumes).

One-year old rhizobia-free seedlings of <u>Acacia</u> <u>confusa</u> and <u>Leucaena leucocephala</u> were prepared from seeds as described in Section 2.53. One month before the assay, one-half of the population was inoculated with rhizobia culture (2.412) while the other half was maintained rhizobia-free. Under greenhouse conditions, the minimum transpiration rate for the seedlings was slightly above 10 ml seedling^{-1} d^{-1}. Therefore, seedlings were irrigated daily with 10 ml of a serial dilution of leachate (73.0, 18.0, 4.56, 1.14, 0.58%) and balanced with distilled water whenever necessary. For each concentration of leachate there were five replications of seedlings. There were two controls: rhizobia-free seedlings receiving N-enriched nutrient solution (Table 2.6) and rhizobia-infected seedlings receiving N-free nutrient solution (Table 2.5). The use of the N-enriched nutrient solution was to provide N for rhizobia-free seedlings. N-free solution was added to infected seedlings. To compare the effects of leachate on legumes and non-legumes, one-year-old seedlings of two non-legumes (Cinnamomum burmanii, Tristania conferta) (1.133) were treated with leachate in the same manner as the two legumes. The control seedlings of non-legumes received N-enriched nutrient solution (Table 2.6). Seedlings of the four species of tree were irrigated with leachate until any seedling could not tolerate the highest concentration and started to die. They were then harvested for biomass analysis and their nodular ARA was measured. The conductivity of aqueous soil extract (2.322) after leachate addition was measured (Table 5.2).

Table 5.1 Physical and chemical characteristics of the Shuen Wan leachate for the irrigation test.

determinand	unit	leachate
pH		8.1
salinity	ppt	10.5
osmolarity	atm	6.85
conductivity	$mS\ cm^{-1}$	19
COD	$mg\ l^{-1}$	655
BOD	$mg\ l^{-1}$	51
NH_4-N	$mg\ l^{-1}$	630
NO_2-N	$mg\ l^{-1}$	0.081
NO_3-N	$mg\ l^{-1}$	0.102
Ni	$mg\ l^{-1}$	0.434
Cu	$mg\ l^{-1}$	0.349
Zn	$mg\ l^{-1}$	0.471
Cd	$mg\ l^{-1}$	0.078

Table 5.2 Conductivity of aqueous soil extract after the leachate treatment. All units in µS cm^{-1}. The conductivity of aqueous extract of Acacia confusa and Leucaena leucocephala soil before the treatment was 56.7 µS cm^{-1} (SD = 22.2); the conductivity of aqueous extract of Cinnamomum burmanii and Tristania conferta initial soil were 25.7 (SD = 1.9) and 8.3 (SD = 0.3) µS cm^{-1}. - = rhizobia-free, + = infected, NA = not applicable.

		legume				non-legume	
species		Acacia confusa		Leucaena leucocephala		Cinnamomum burmanii	Tristania conferta
rhizobia infection		+	-	+	-	NA	NA
leachate concentration (%)							
control	mean	59.5	115.1	51.3	120.0	35.3	24.2
	SD	2.7	18.0	3.7	30.2	1.4	2.5
0.58	mean	39.5	50.6	55.1	39.2	25.6	10.3
	SD	14.8	14.7	26.0	15.6	2.1	0.4
1.14	mean	28.0	40.0	19.0	39.2	22.0	13.0
	SD	2.6	3.7	1.7	9.5	0.6	0.3
4.56	mean	59.7	55.0	82.1	56.1	35.8	22.3
	SD	5.5	7.8	15.0	4.7	6.7	0.8
18.0	mean	117.5	149.9	162.4	104.5	83.5	43.1
	SD	26.7	43.2	14.1	13.4	3.7	1.0
73.0	mean	387.0	357.0	370.0	366.4	283.0	266.7
	SD	22.5	56.3	26.5	315.3	5.0	5.9

5.3 Effect on legumes

The influence of landfill leachate was studied by the addition of a series of leachate on seedlings of two legumes. Negative photosynthetic rate (net respiration) (Fig. 5.1) was found in some seedlings of both legumes at the highest concentration of leachate after a five-month period. Some of their leaves became brittle and the apex became dark brown; the test was therefore terminated and all the seedlings were harvested. However, some rhizobia-infected Leucaena leucocephala seedlings at the highest concentration of leachate (73.0%) carried out photosynthesis at similar rate as those seedlings at lower concentrations of leachate. Photosynthetic rate of the compound leaves of the rhizobia-free L. leucocephala seedlings at the highest concentration of leachate was not measured as most of their leaves were dropped.

For both species of legume, infected seedlings had higher total biomass (5 - 60%) than rhizobia-free seedlings at the same concentration of leachate, with the exception of Acacia confusa at the lowest concentration of leachate (Figs 5.2, 5.3). The highest biomass was found at 18.0% leachate.

The ARA of Leucaena leucocephala seedlings at ≥ 1.14% leachate was hardly detectable (Fig. 5.6) (either free of nodules or the C_2H_4 concentration was beyond the detection limit of the gas chromatograph (2.7)). Acacia confusa nodules at 4.56 and 18.0% leachate showed similar ARA (60 and 75%) to the control; ARA was non-detectable at the lowest leachate concentration and in the two highest leachate concentrations (73.0, 18.0%).

The macronutrient contents (N, P, K) of the two legumes were compared. The total N content in roots of the rhizobia-infected seedlings was generally higher than in rhizobia-free seedlings, especially on A. confusa. However, the total N content in the stems and leaves of rhizobia-free seedlings was generally higher than in the infected seedlings. The total P content in rhizobia-infected A. confusa seedlings was higher than the rhizobia-free seedlings. No difference was found in Leucaena leucocephala. No difference was found in the total K content between rhizobia-free and infected seedlings of both species (Figs 5.8, 5.9).

No detectable increase in metal concentrations (Ni, Cu, Zn, Cd) was found after leachate addition (Figs 5.10 to 5.13). The metal conentrations in both legumes were of the similar ranges. Nodulation did not affect the metal concentrations in the plant tissue.

5.4 Effect on non-legumes

Seedlings of the two non-legumes at the higher leachate concentrations (> 4.56%) showed abnormal growth after one-month. Their leaves became brittle, started to drop and a negative photosynthetic rate was found (Fig. 5.1); all seedlings therefore were harvested.

For both non-legumes, the biomass of different plant parts at the three medial concentrations of leachate (18.0, 4.56 and 1.14%) was higher than at the highest (73.0%) or lowest concentration (0.58%) (Figs 5.4, 5.5).

The total N content in tissue of the two non-legumes at high leachate concentrations was generally higher than at low leachate concentrations (Fig. 5.7). The total N content in the non-legumes was lower than in the legumes (5.3).

Leachate addition did not affect the total P and total K content in the non-legumes (Figs 5.8, 5.9). No detectable increase in metal concentrations (Ni, Cu, Zn, Cd) was found (Figs 5.10 to 5.13). Metal concentrations in Tristania conferta was generally slightly higher than in Cinnamomum burmannii, except Cd.

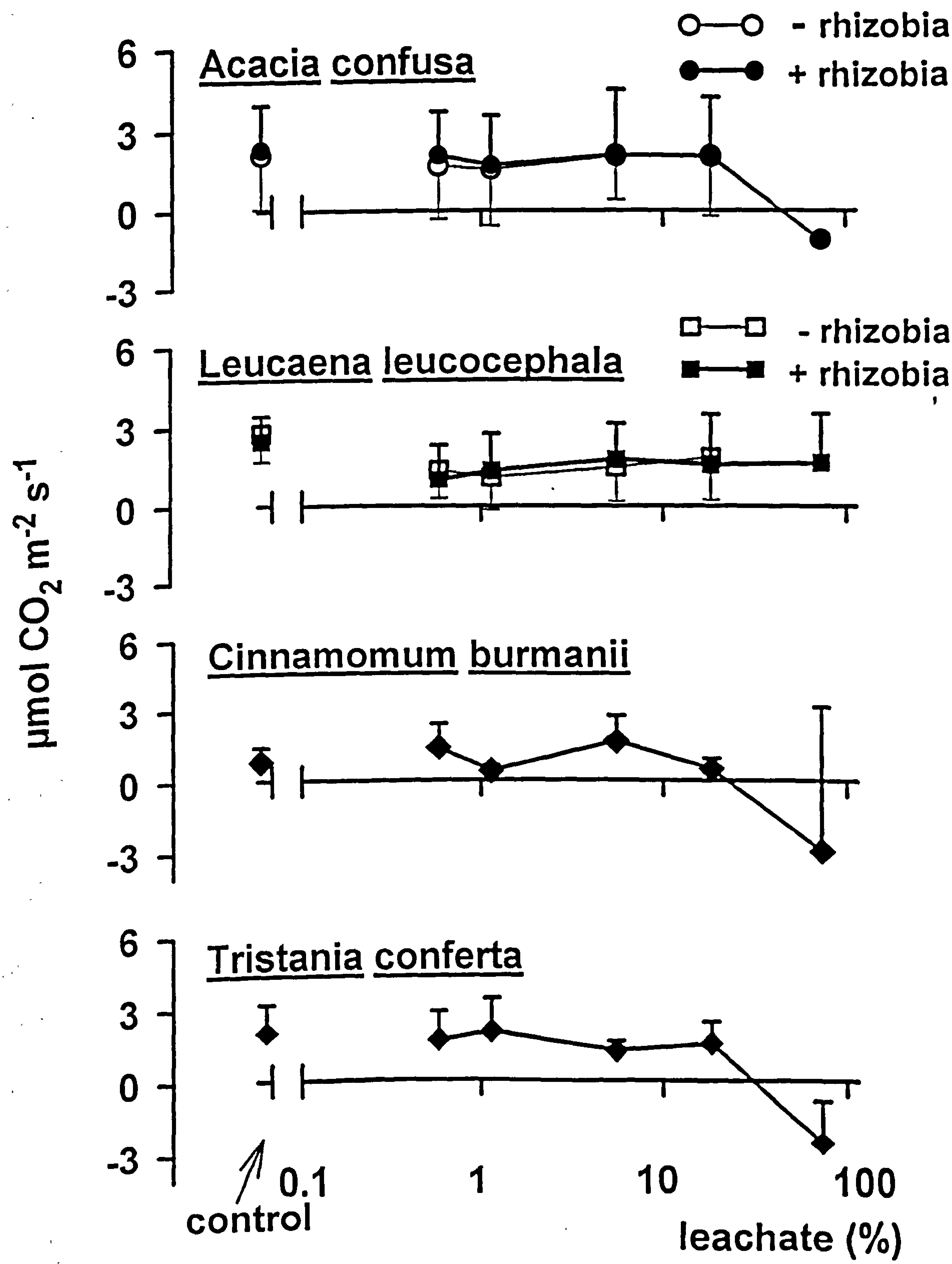


Fig. 5.1 Influence of leachate on leaf photosynthesis. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.

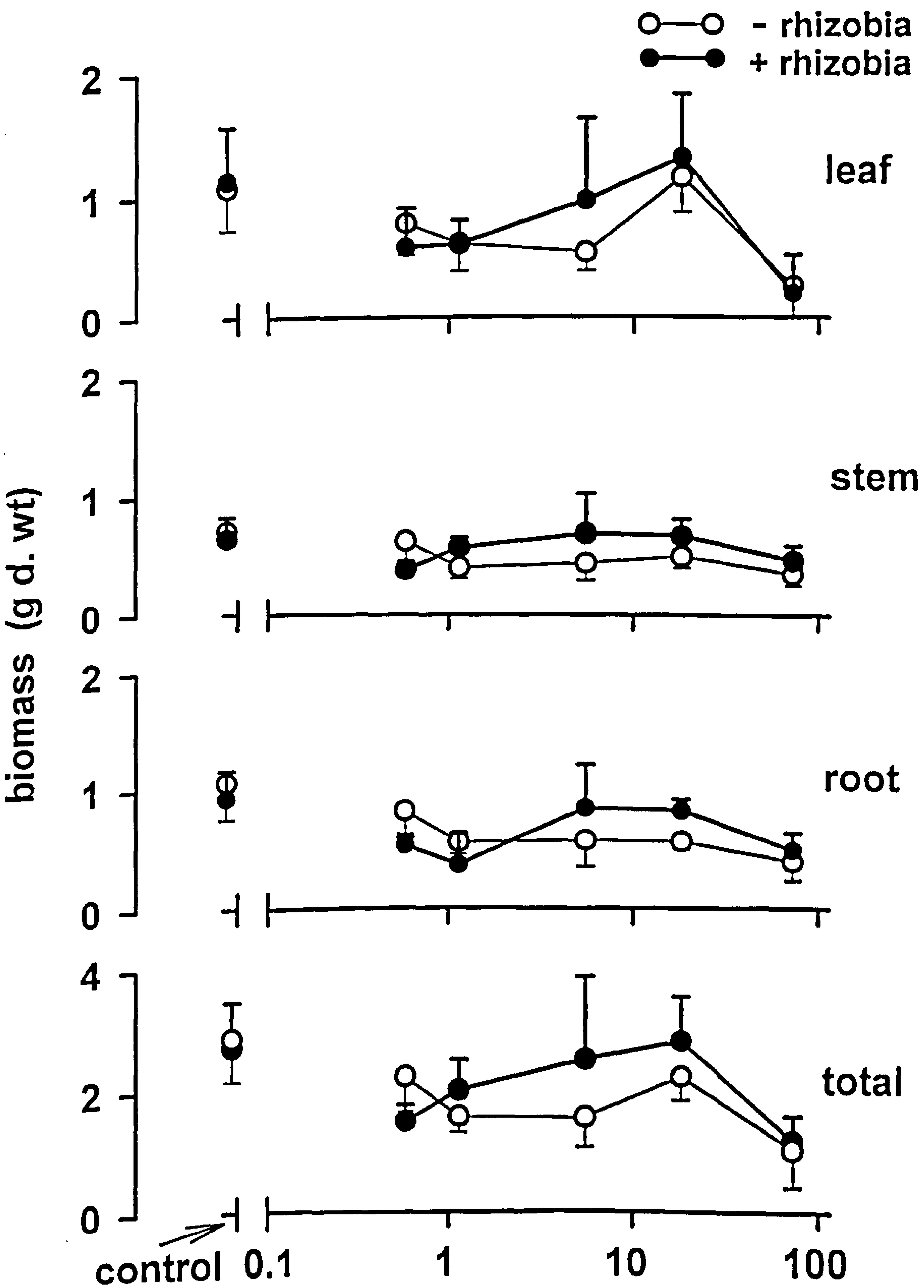


Fig. 5.2 Influence of leachate on Acacia confusa biomass. Seedlings were irrigated with a serial dilution of leachate for five months.

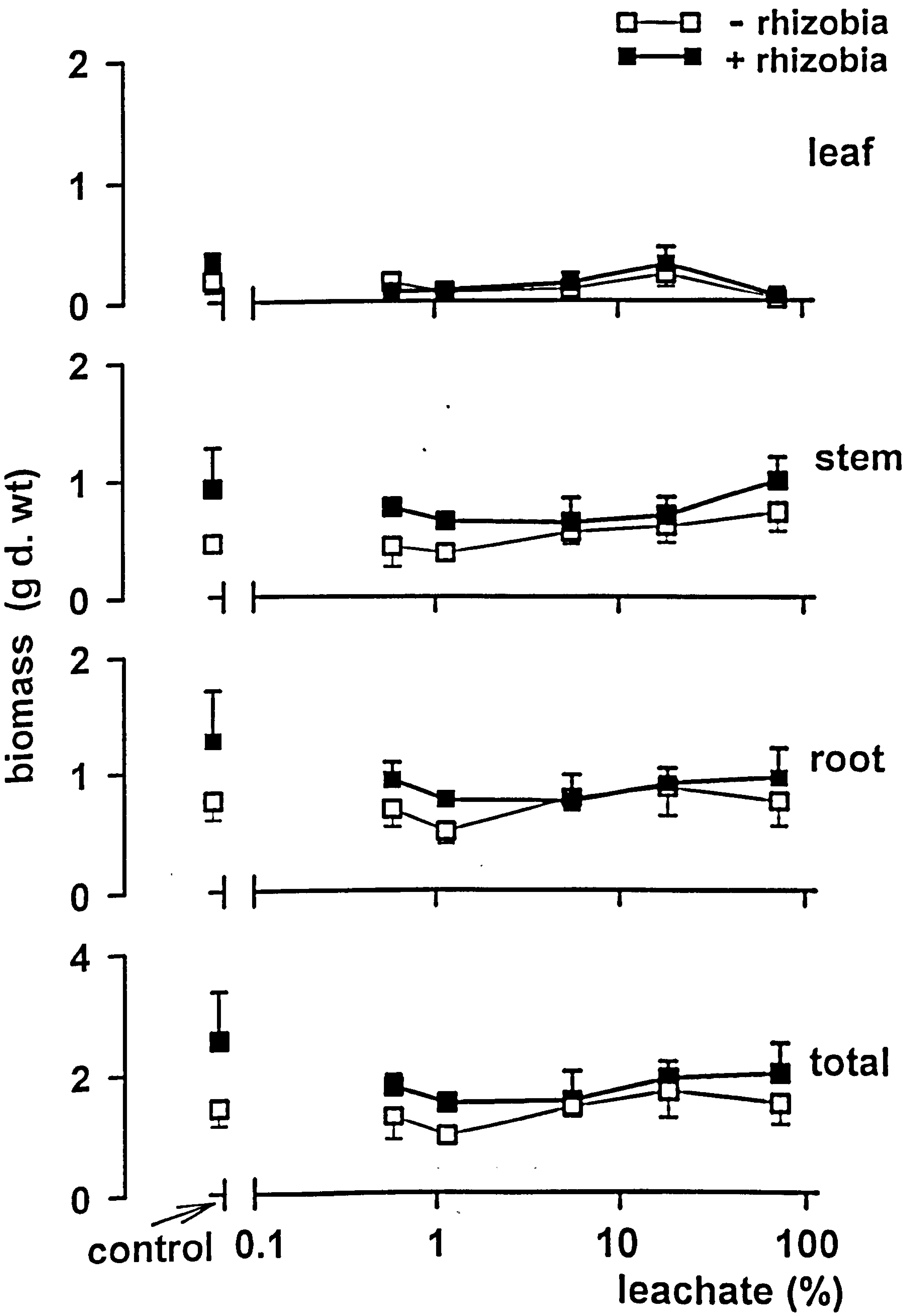


Fig. 5.3 Influence of leachate on Leucaena leucocephala biomass. Seedlings were irrigated with a serial dilution of leachate for five months.

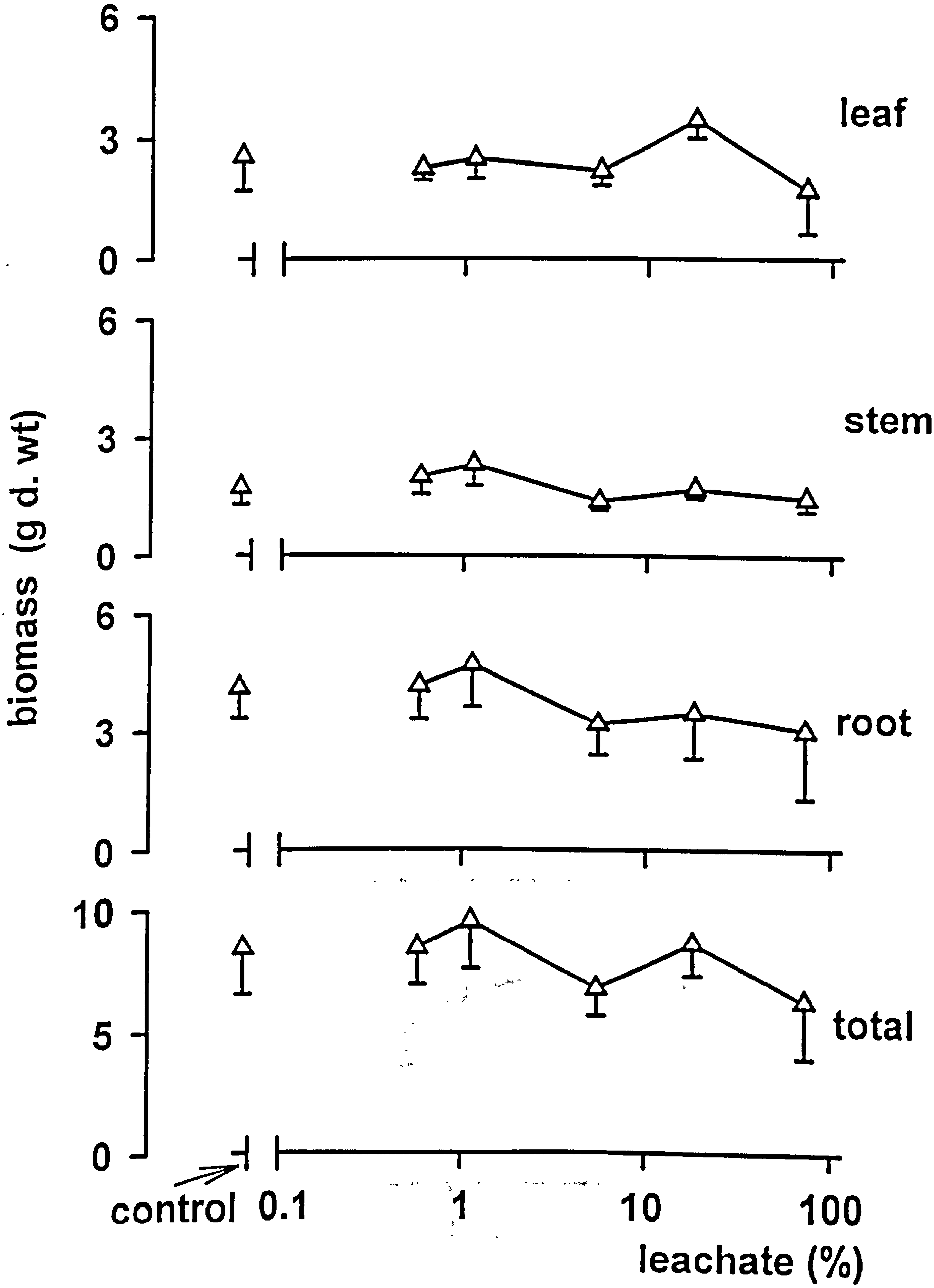


Fig. 5.4 Influence of leachate on Cinnamomum burmanii biomass. Seedlings were irrigated with a serial dilution of leachate for one month.

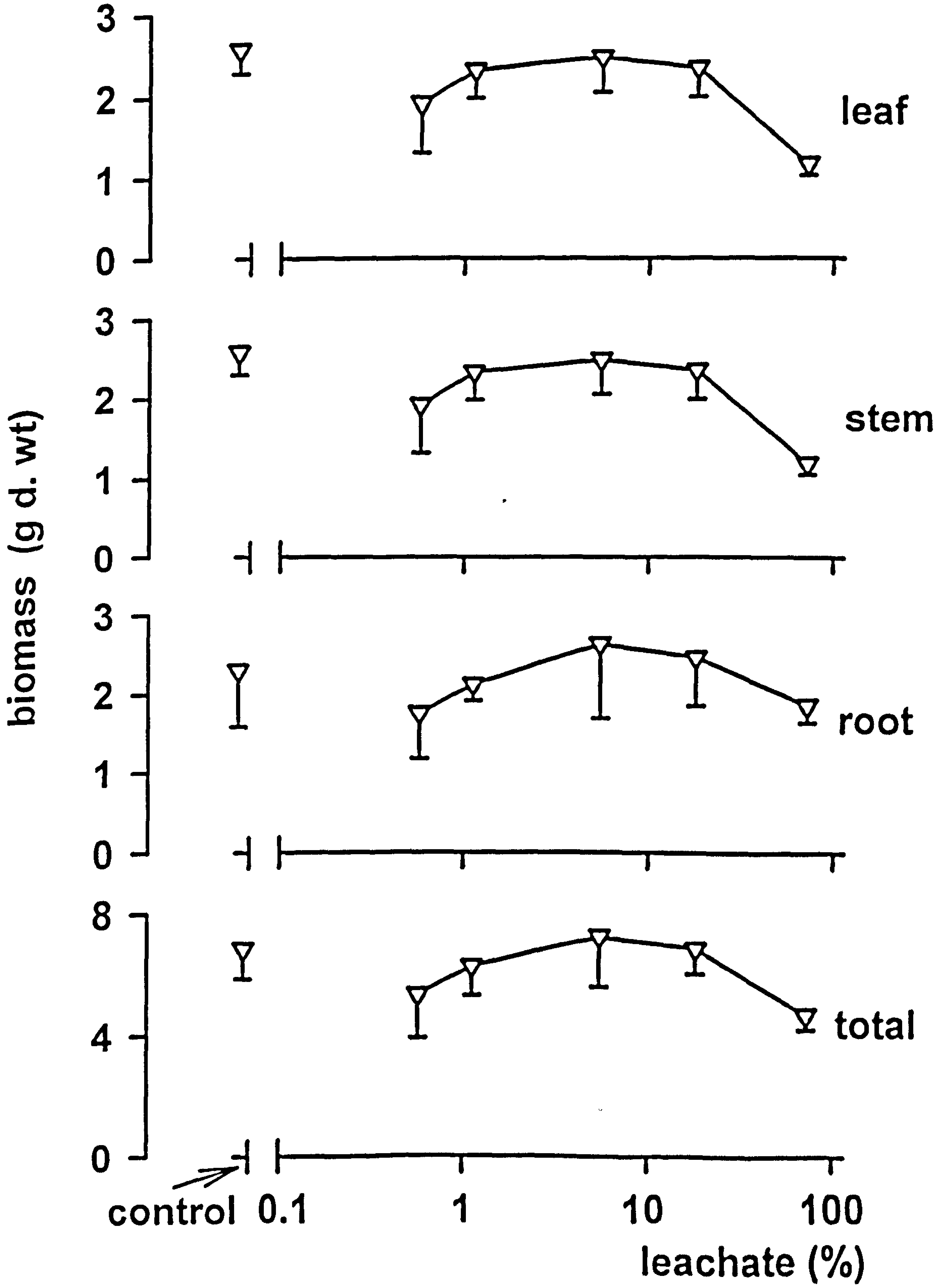


Fig. 5.5 Influence of leachate on Tristania conferta biomass. Seedlings were irrigated with a serial dilution of leachate for one month.

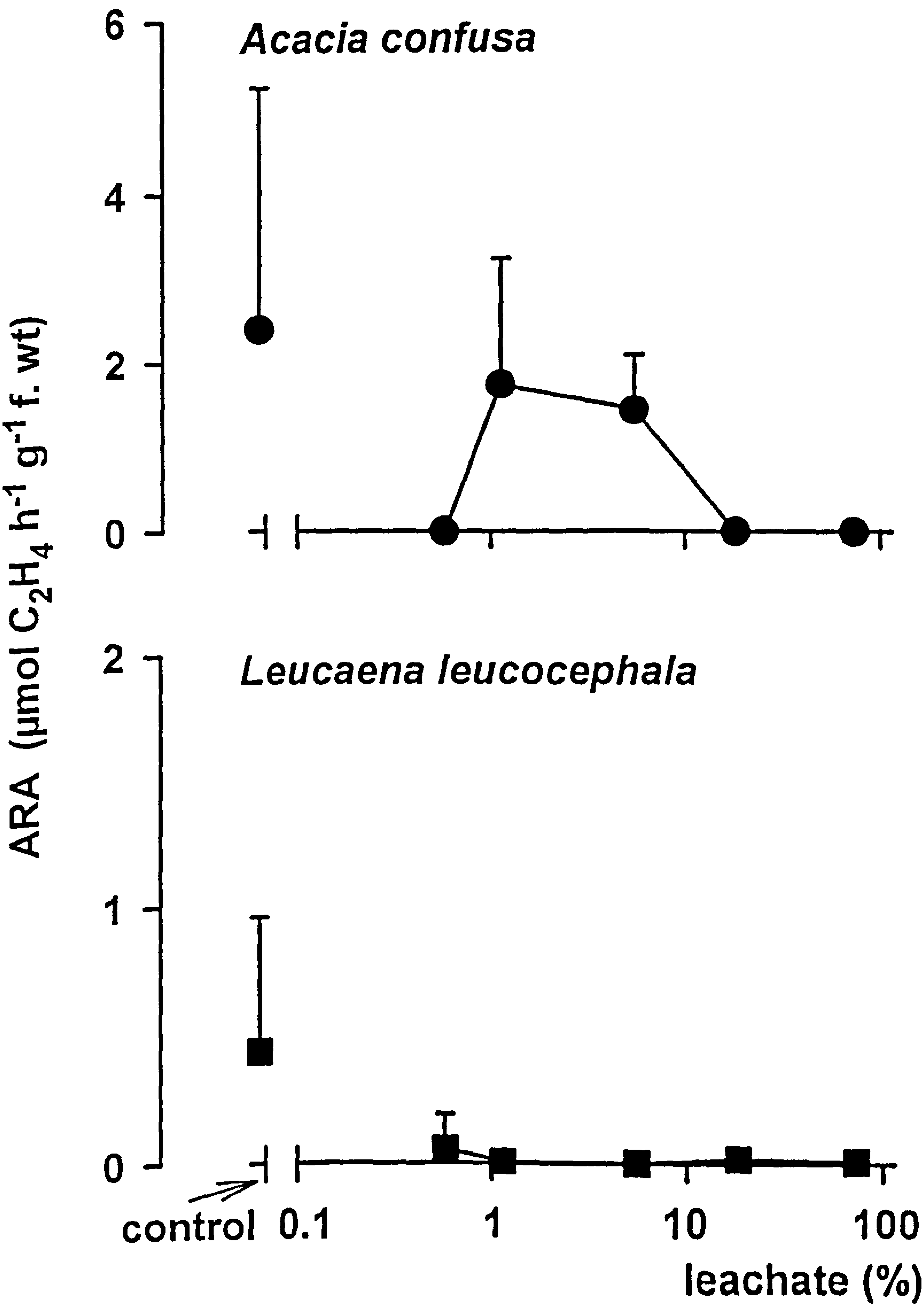
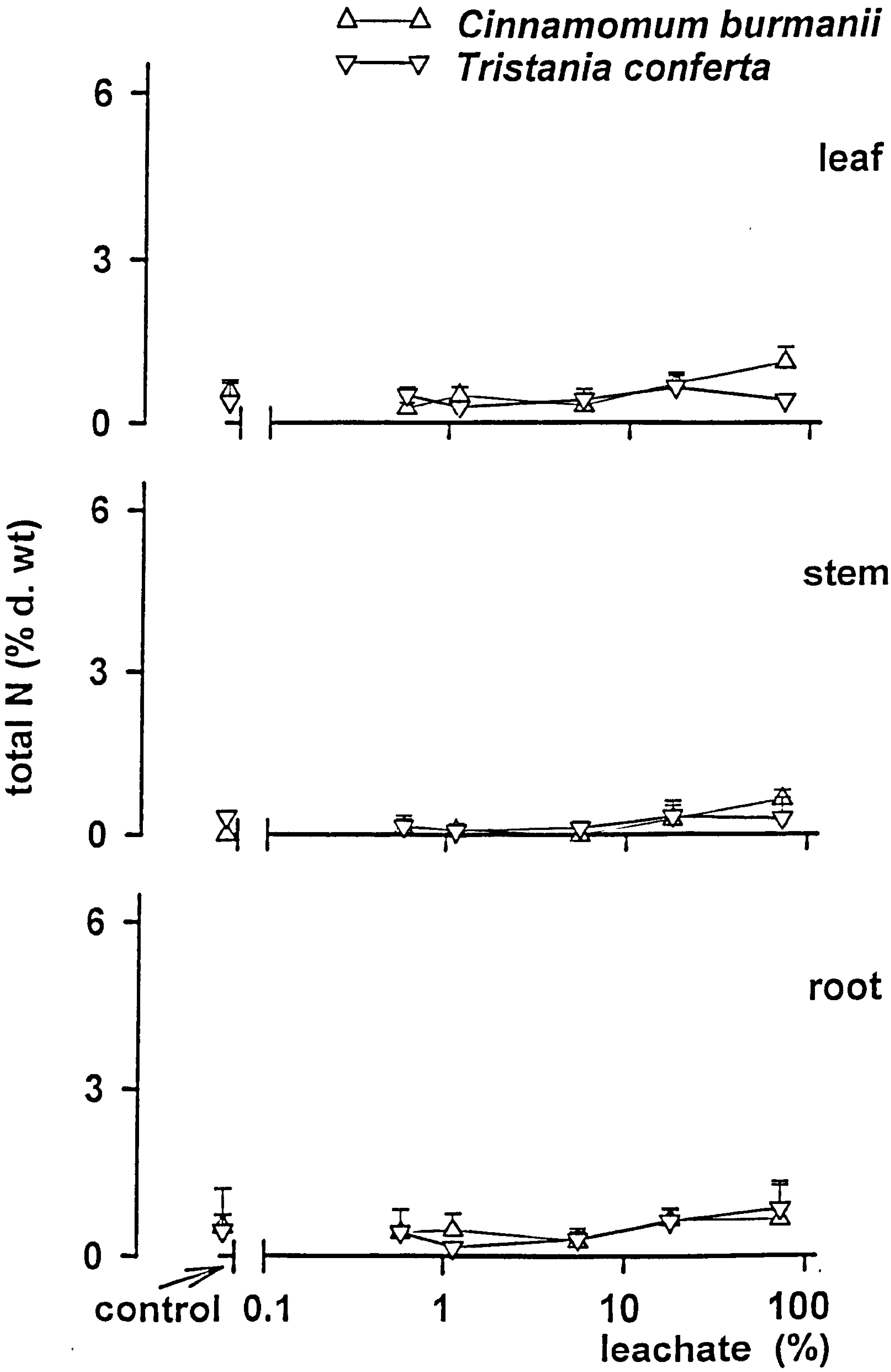


Fig. 5.6 Influence of leachate on ARA of nodules. Seedlings were irrigated with a serial dilution of leachate for five months.


Cinnamomum burmanii
Tristania conferta
leaf
stem
root
total N (% d. wt)
6
3
0
control
0.1
1
10
100
leachate (%)

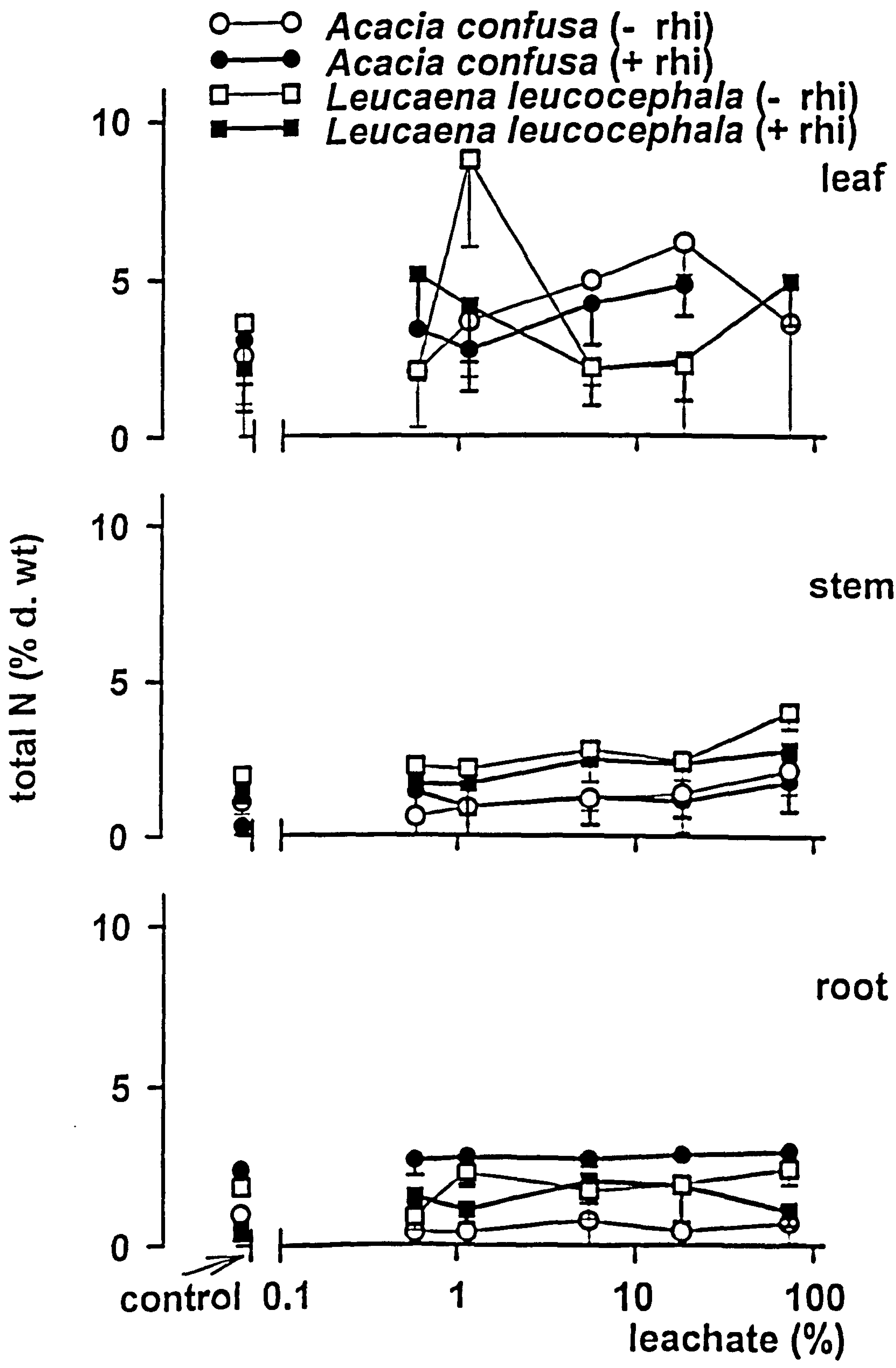
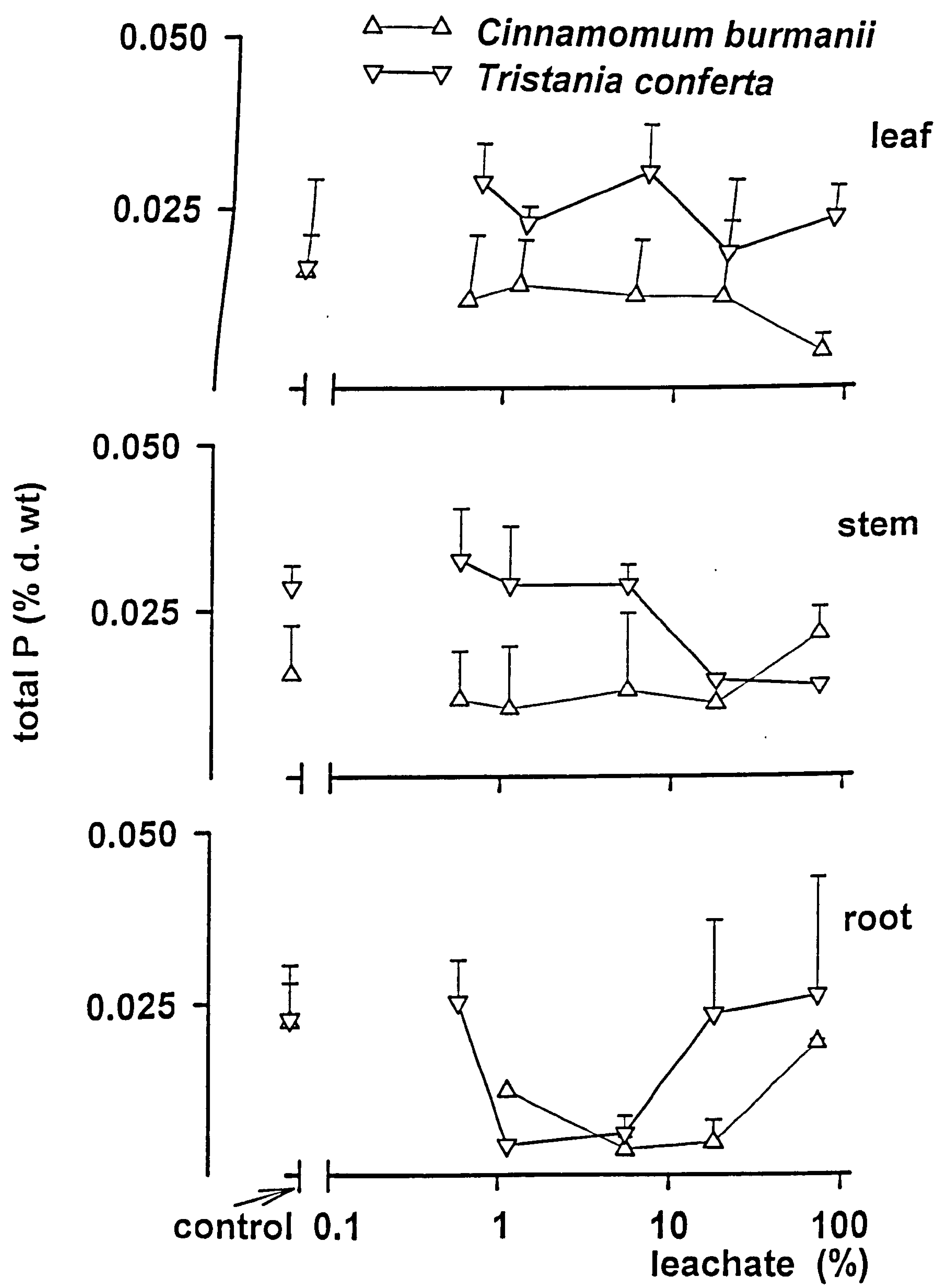


Fig. 5.7 Influence of leachate on total N content of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.



Cinnamomum burmanii
Tristania conferta
leaf
stem
root
0.050
0.025
total P (% d. wt)
control
0.1
1
10
100
leachate (%)

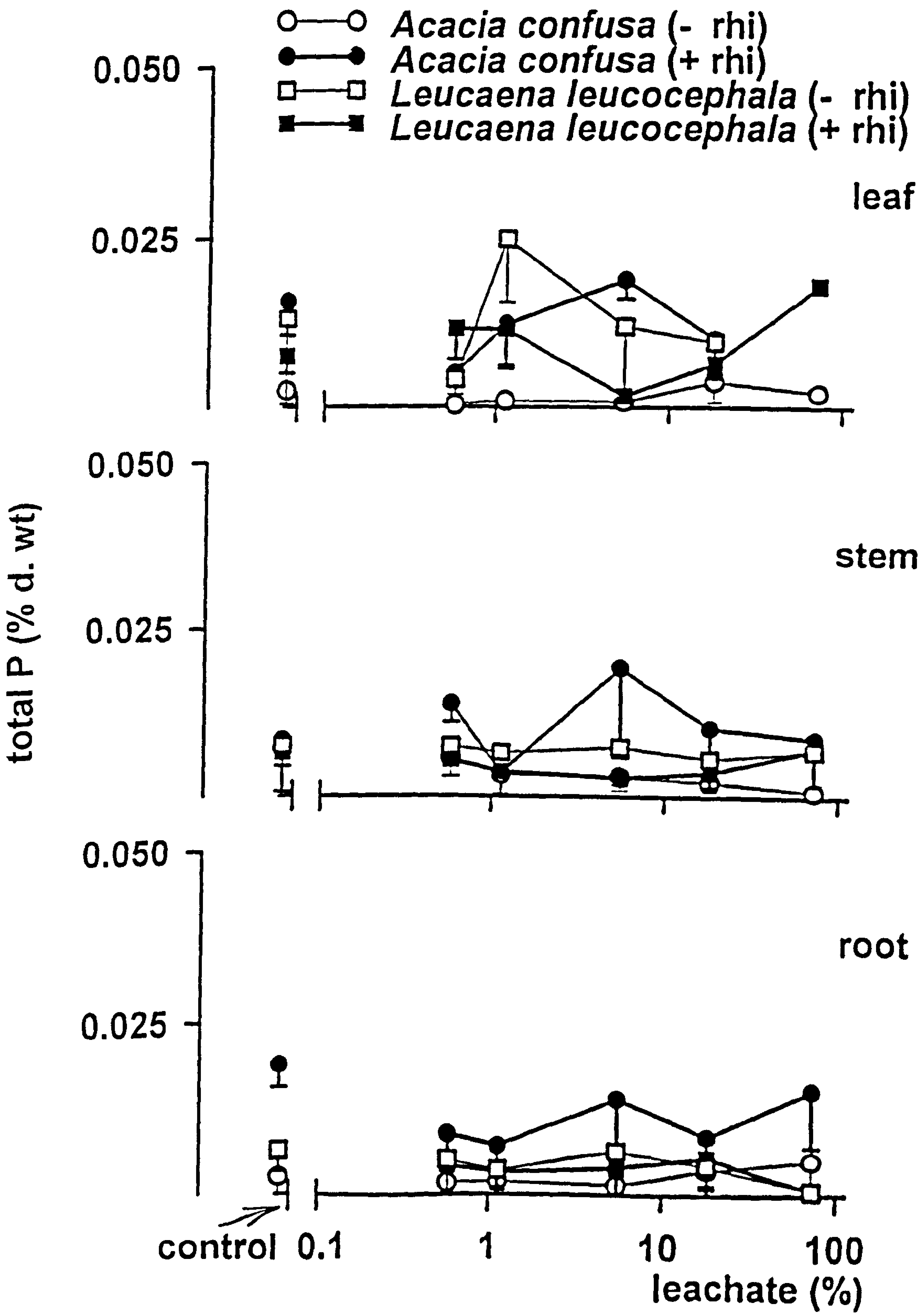
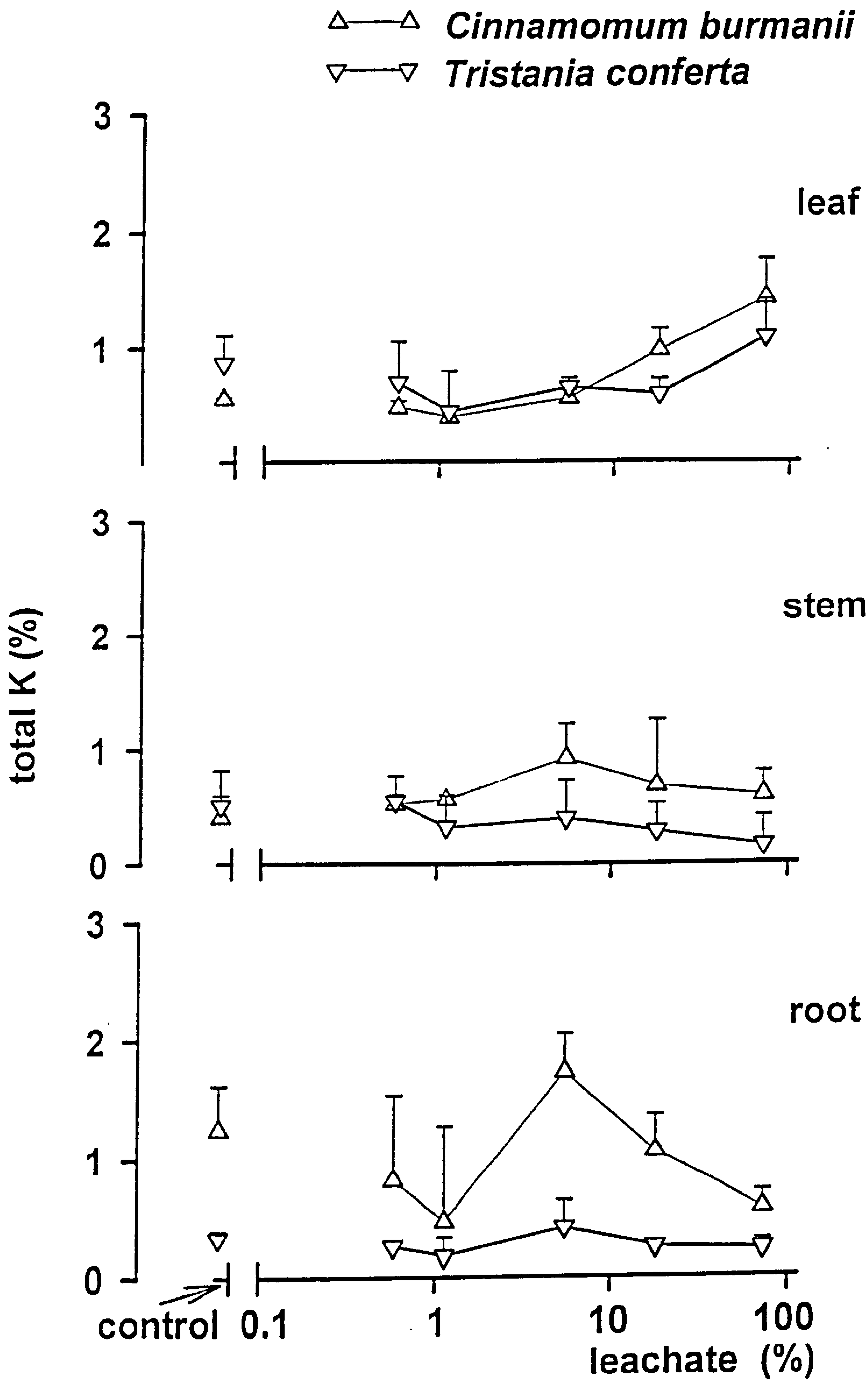


Fig. 5.8 Influence of leachate on total P content of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.


Cinnamomum burmanii
Tristania conferta
leaf
stem
root
total K (%)
0
1
2
3
control
0.1
1
10
100
leachate (%)

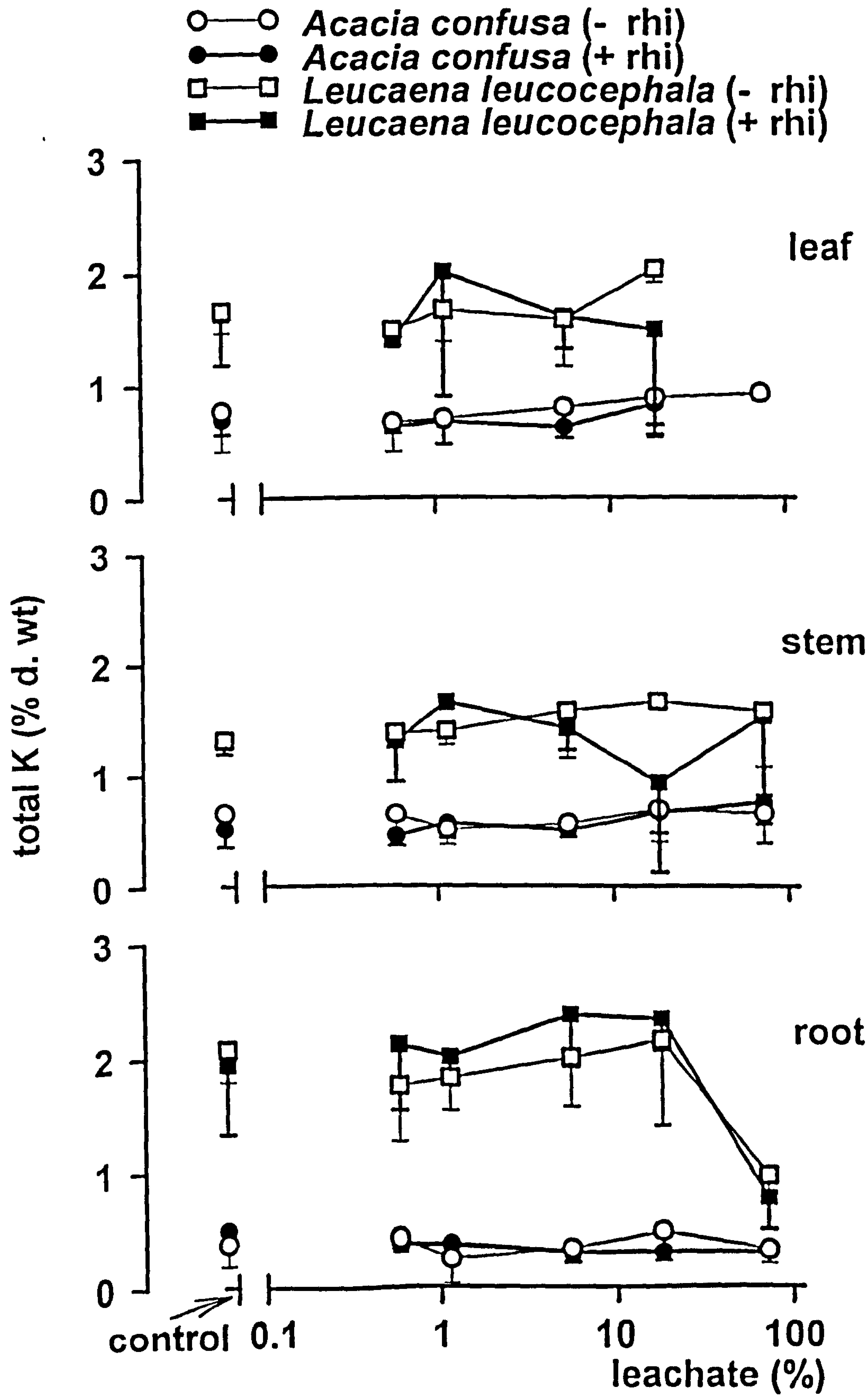
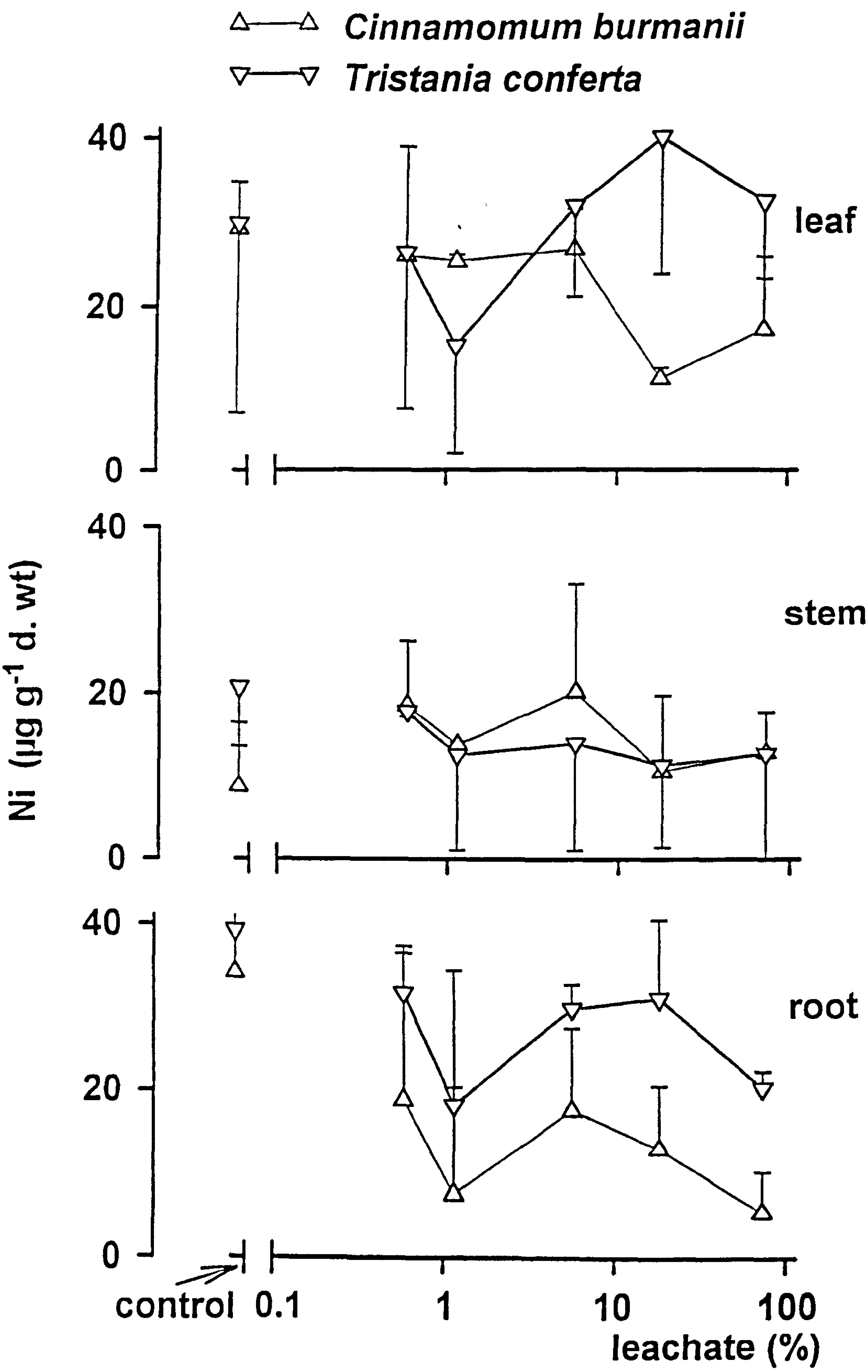


Fig. 5.9 Influence of leachate on total K content of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.


Cinnamomum burmanii
Tristania conferta
40
20
0
leaf
stem
root
Ni (µg g-1 d. wt)
control
0.1
1
10
100
leachate (%)

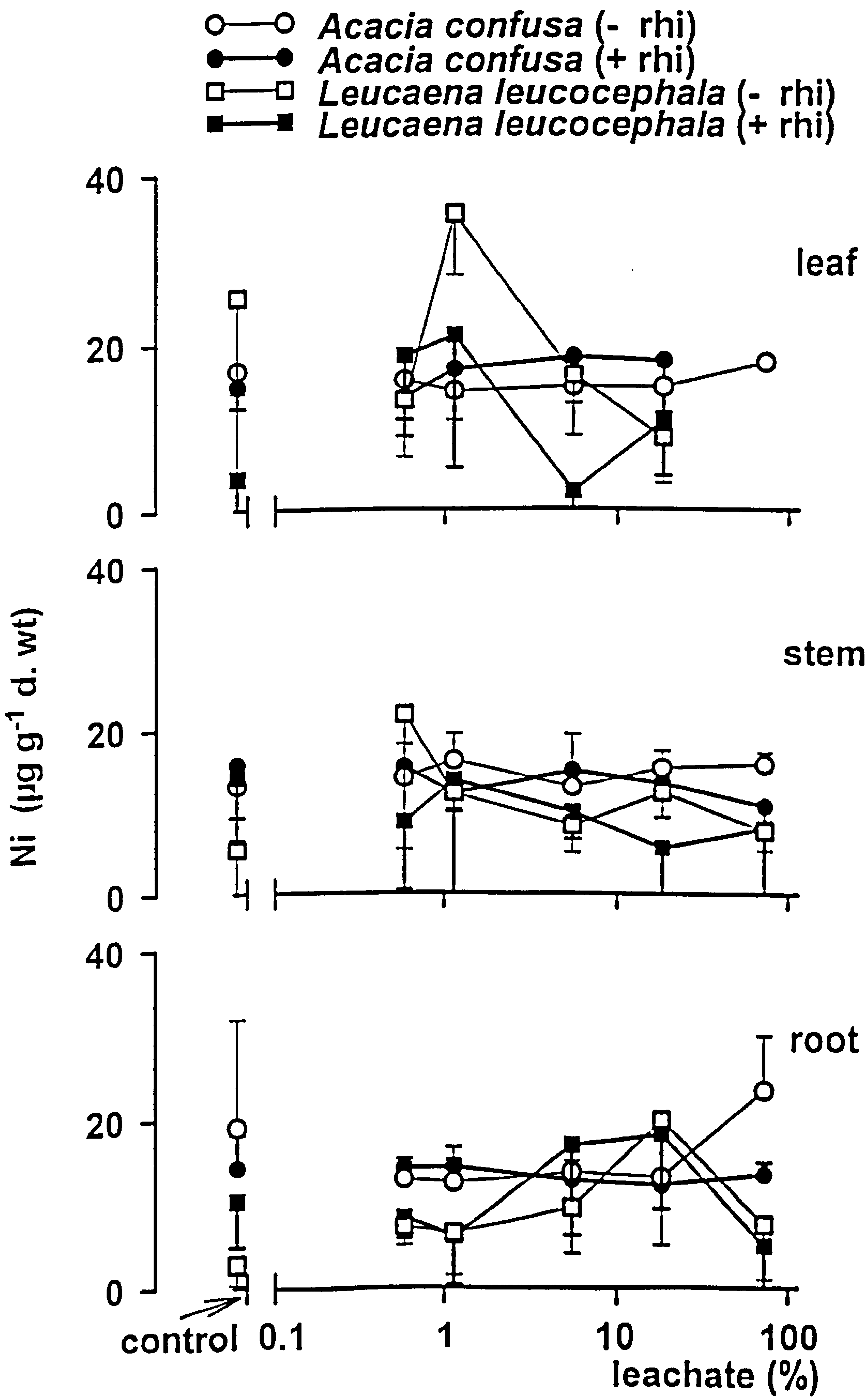
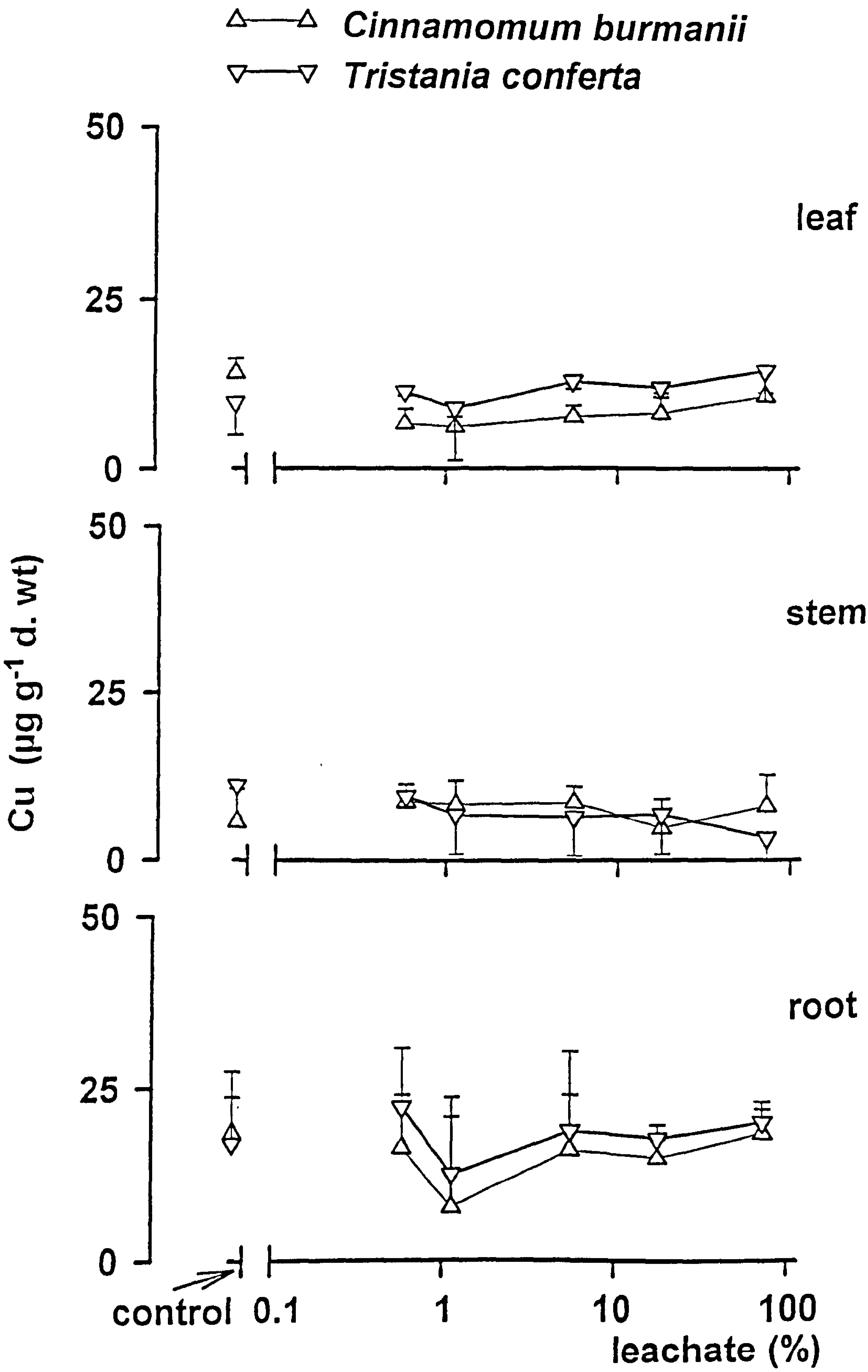


Fig. 5.10 Influence of leachate on Ni concentration of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.


Cinnamomum burmanii
Tristania conferta
50
25
0
leaf
50
25
0
stem
Cu (µg g-1 d. wt)
50
25
0
root
control
0.1
1
10
100
leachate (%)

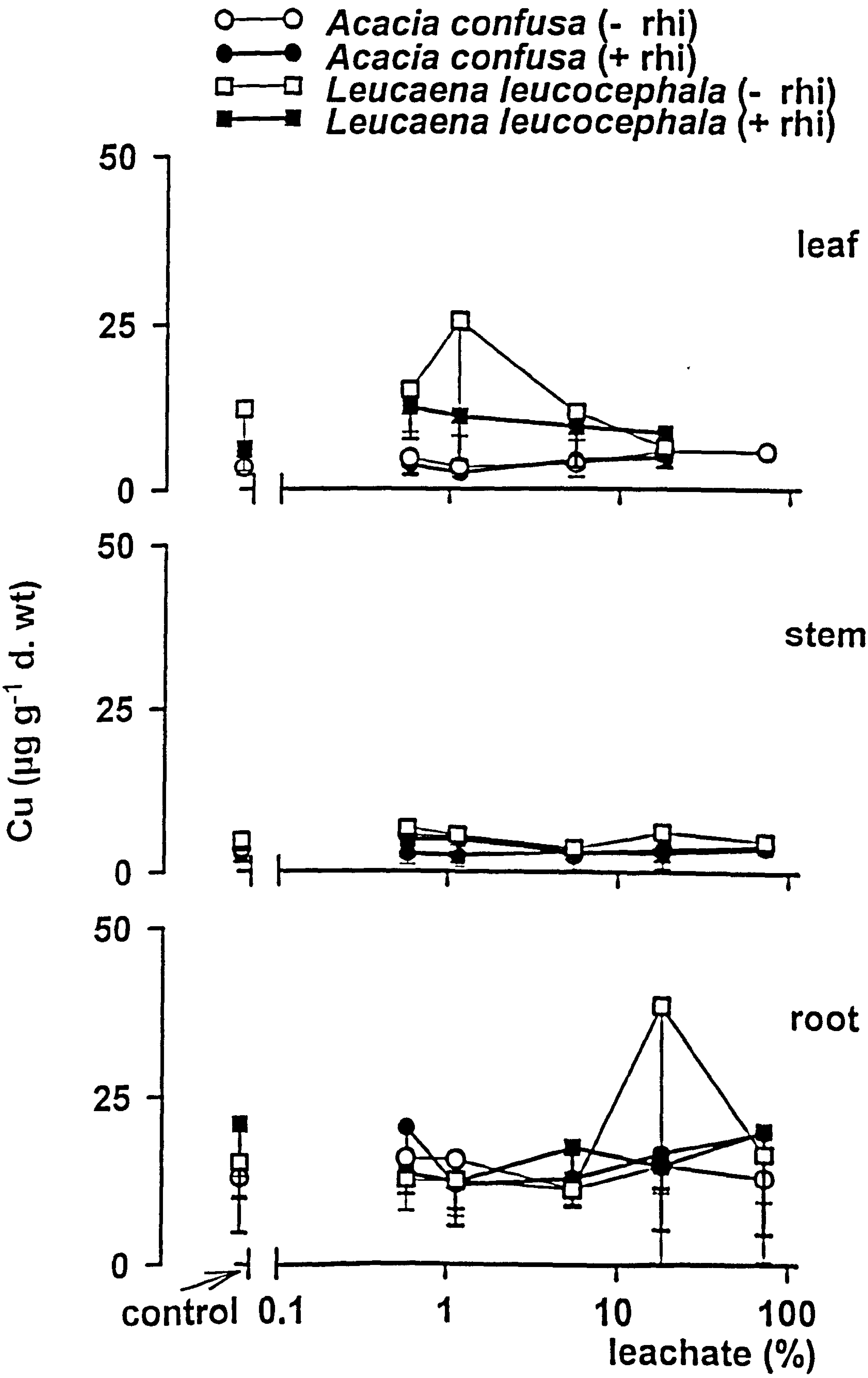
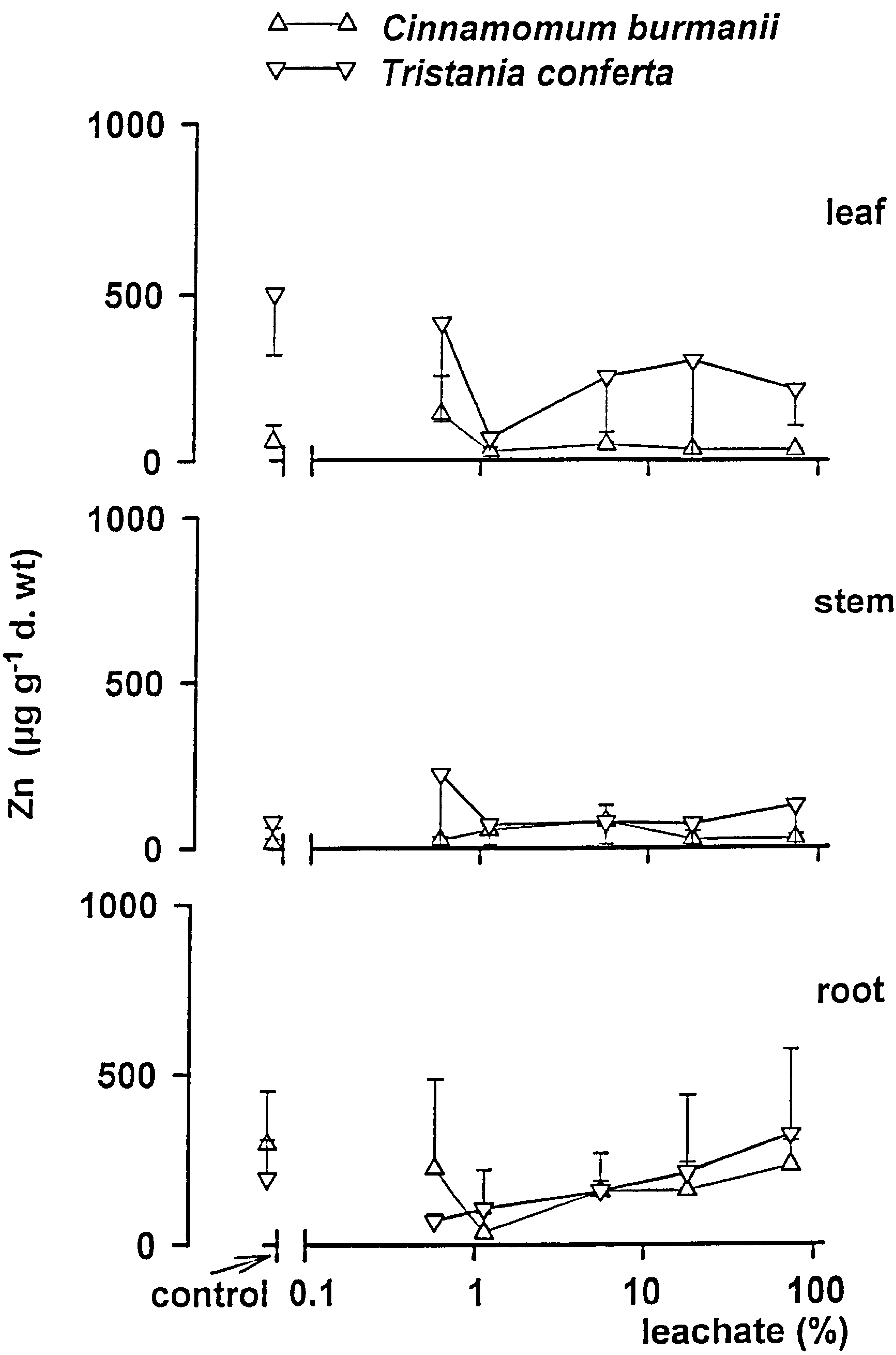


Fig. 5.11 Influence of leachate on Cu concentration of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.


Cinnamomum burmanii
Tristania conferta
1000
500
0
leaf
stem
root
Zn (µg g-1 d. wt)
control
0.1
1
10
100
leachate (%)

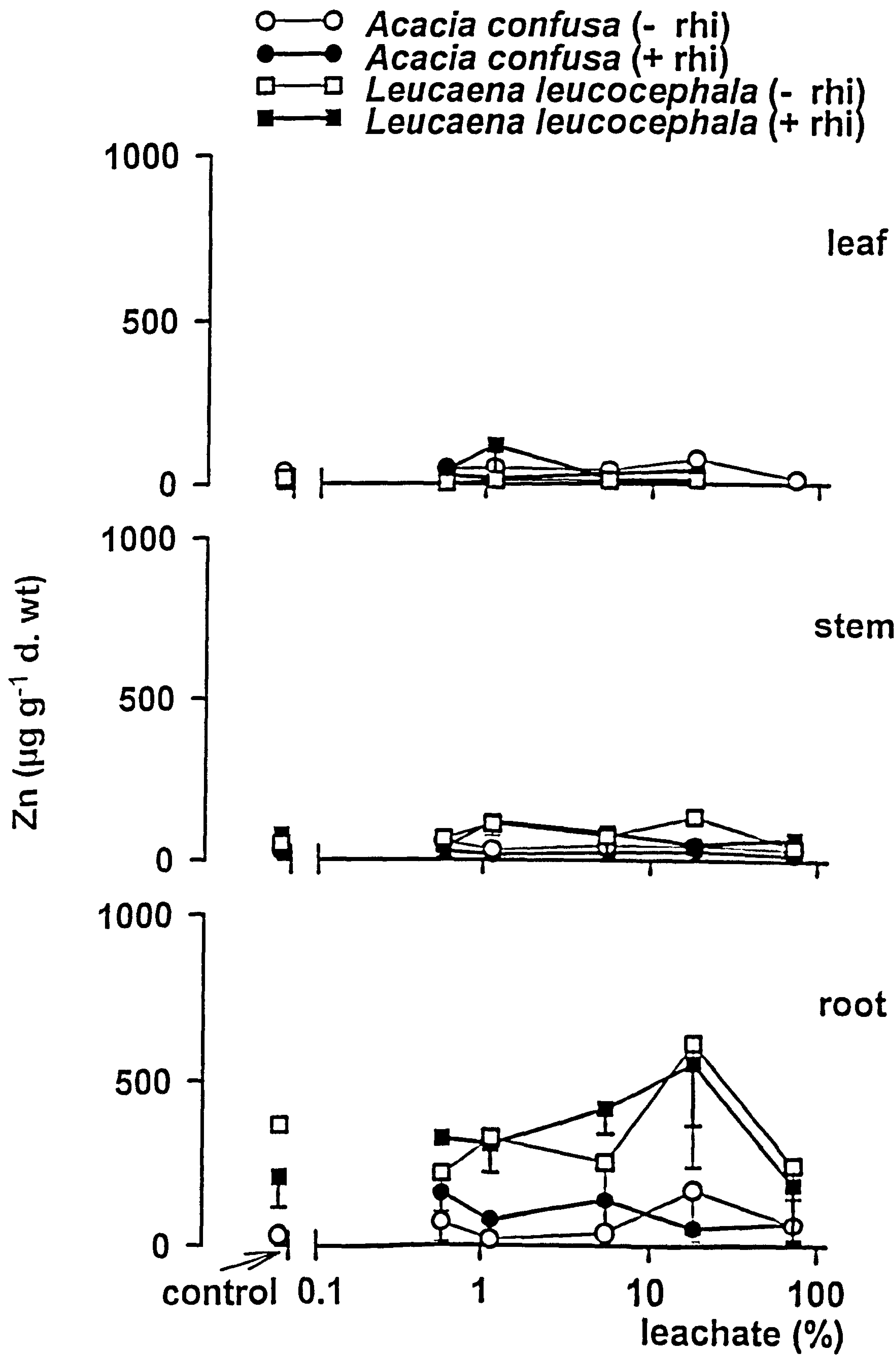
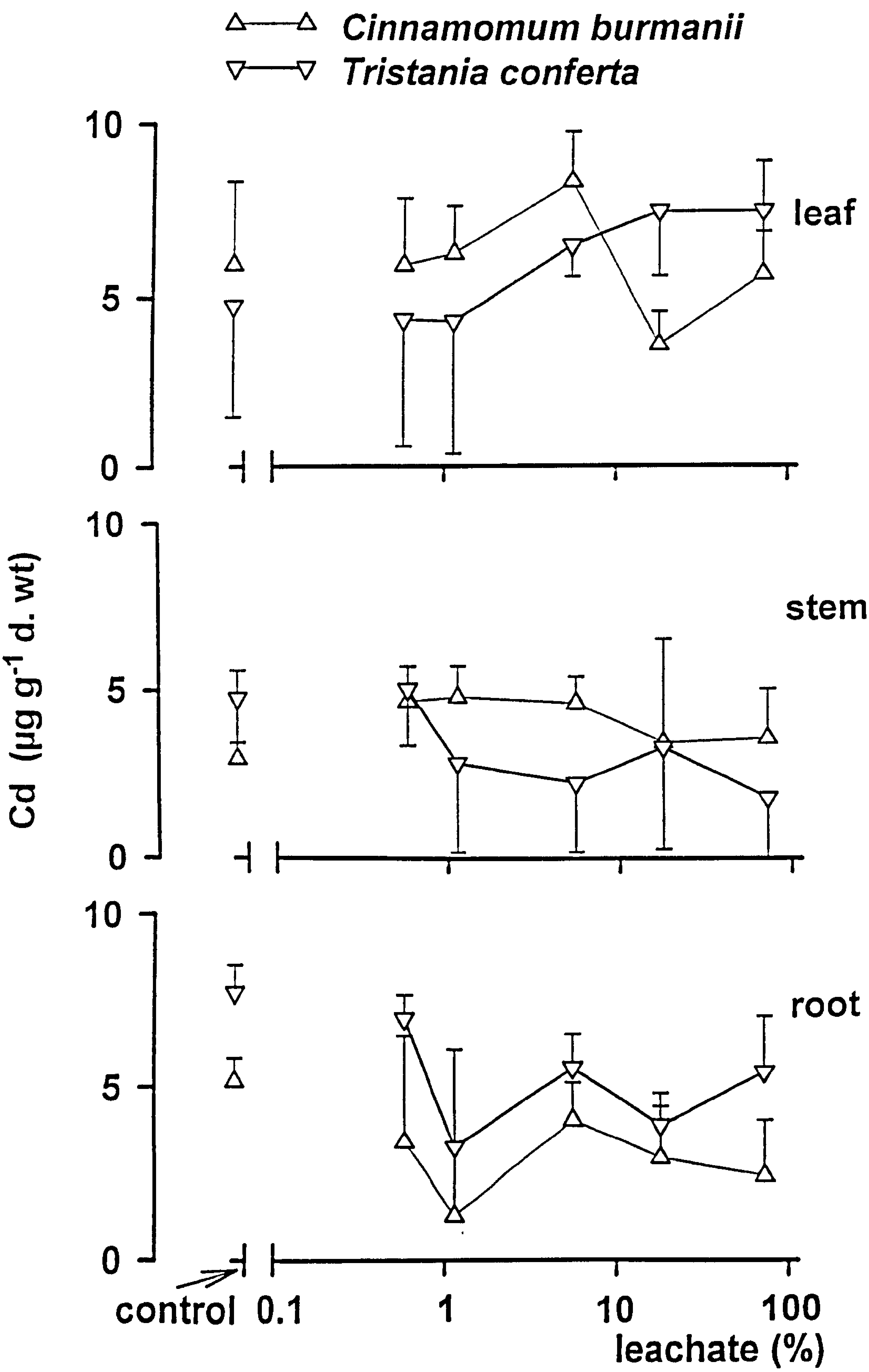


Fig. 5.12 Influence of leachate on Zn concentration of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.


Cinnamomum burmanii
Tristania conferta
leaf
stem
root
Cd (µg g-1 d. wt)
10
5
0
control 0.1
1
10
100
leachate (%)

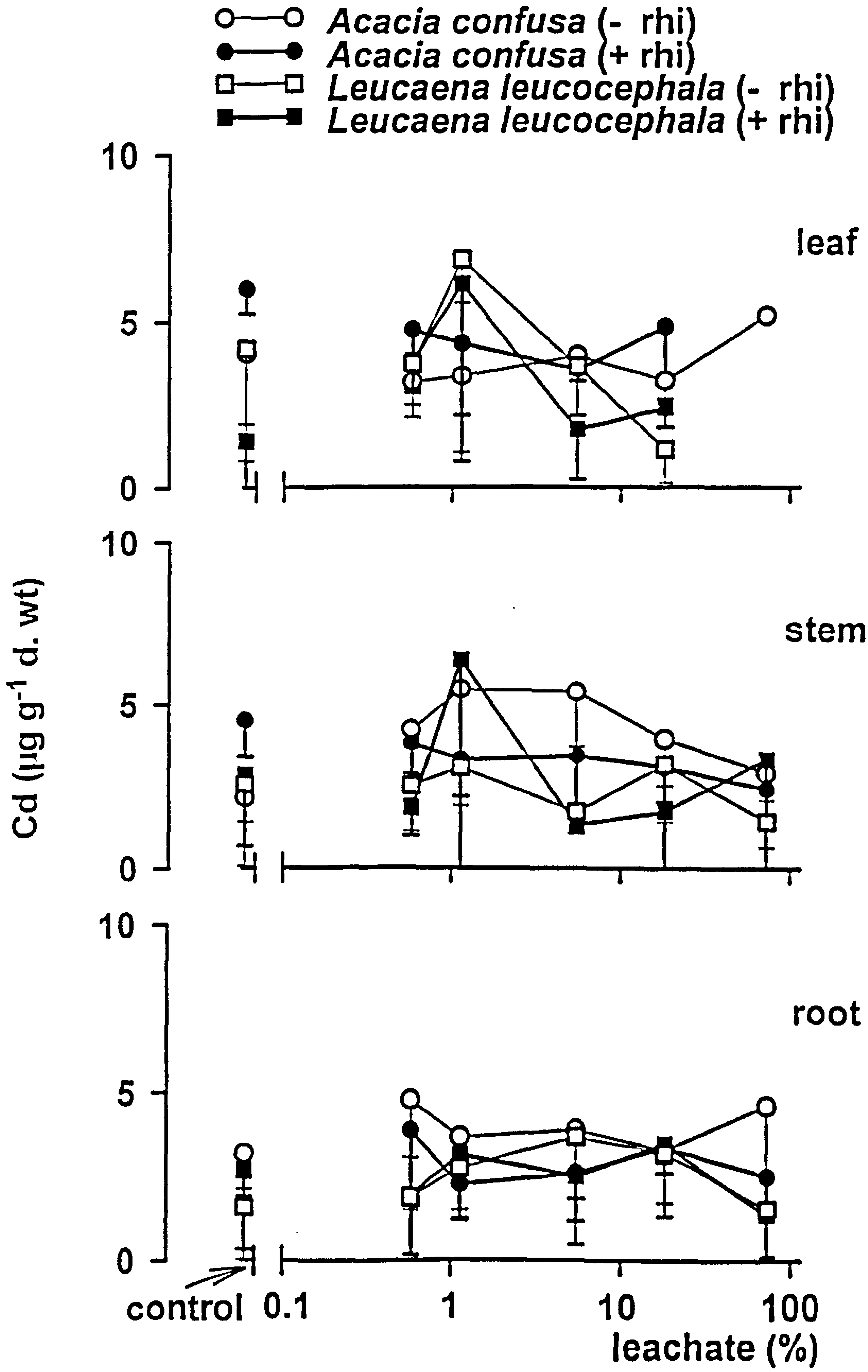


Fig. 5.13 Influence of leachate on Cd concentration of two legumes and two non-legumes. Seedlings of Acacia confusa and Leucaena leucocephala were irrigated with a serial dilution of leachate for five months. Cinnamomum burmanii and Tristania conferta were irrigated with leachate for one month.

CHAPTER 6
FIELD TRIAL

6.1 Overview

The results of Chapter 4 indicate that landfill gas can be either stimulatory or inhibitory to growth and N_2 fixation of Acacia confusa and Leucaena leucocephala and their associated nodules, depending on the concentrations of the individual gas and the period of exposure. Both legumes can tolerate high concentrations of leachate in soil (Chapter 5), but the ARA of their nodules would be inhibited at such concentrations. However, the above results were based on experiments conducted under laboratory and greenhouse conditions. To verify the above results, a field trial was conducted on two landfill sites. The availability of free rhizobia to infect legumes was tested and the growth and N_2 fixation of the legumes and their associated nodules was assessed.

6.2 Field site and assay method

Shuen Wan and Junk Bay Stage I, II/III were chosen for the field trials. These sites had been operated for more than ten years. Compared with other sites, the gaseous levels at these sites were higher and suited for field study on the influence of landfill factors. Areas which had already been covered by a permanent clay cap for at least two years and where no more waste was to be laid, were chosen for the trials. Other details of the sites are given in Sections 1.132 and 3.3 to 3.5.

Ten-month old rhizobia-free seedlings of Acacia confusa and Leucaena leucocephala were germinated (2.53) and planted under greenhouse conditions. 40 healthy seedlings of each species were transplanted to the Shuen Wan Landfill and 20 seedlings were transplanted to each of the Junk Bay I and II/III sites in August 1993; one half of the seedling population were rhizobia-free (the rhizobia-free group) and others were inoculated artificially with rhizobia (the pre-inoculated group) (2.412) two weeks before the transplantation. As the Junk Bay Stage I and Stage II/III were only about

400 m apart and all field trial work was conducted on the same days, the data collected on both sites were analyzed collectively and reported as Junk Bay Landfill.

6.3 Can rhizobia infect on legume under landfill condition?

All transplanted seedlings were harvested after six-months of growth on the landfill sites. Most (17 to 89%) of the rhizobia-free seedlings became inoculated (Table 6.1) and the nodules could be identified by naked eye. The maximum depth of the root system of seedlings established on the sites was 20 cm. (Gaseous composition in topsoil was measured at 35 to 50 cm from soil surface (2.21).) Nodules were found generally to be on the upper zone of the root system. The inoculation rates at Junk Bay (70, 89%) were generally higher than at Shuen Wan (17, 63%).

6.4 Influence of rhizobial infection for legumes on the completed landfill site

The harvested nodule f. wt and ARA (h^{-1} g^{-1} f. wt and $plant^{-1}$) of nodules in the pre-inoculated group were generally higher than in the rhizobia-free group. The total biomass of the pre-inoculated seedlings was 20% to 130% higher than the seedlings of the rhizobia-free group, except Acacia confusa at Shuen Wan (15% less).

The relationship between ARA and harvested biomass was tested at different sites and between the two treatment groups (Table 6.2). The coefficient of correlation between ARA ($plant^{-1}$) and the leaf biomass of the pre-inoculated seedlings ($r = 0.529$ to 0.853, $P = 0.05$) was generally higher than the coefficient of correlation between ARA and leaf biomass of seedlings which were rhizobia-free when transplanted to sites ($r = 0.171$ to 0.742, $P = 0.05$). Moreover, the coefficient of correlation between the leaf and nodule biomass ($r > 0.6$, $P = 0.05$, except for Acacia confusa at Shuen Wan) was generally higher than the coefficient of correlation between the leaf biomass and ARA.

Table 6.1 Inoculation rates, ARA and harvest biomass of legume seedlings, after six-month of growth at landfill sites. (- rhi: seedlings were rhizobia-free when transplanted to sites; + rhi: seedlings were inoculated artificially when transplanted to sites; n = 20).

legume		Acacia confusa				Leucaena leucocephala			
landfill site		Junk Bay		Shuen Wan		Junk Bay		Shuen Wan	
transplanted		- rhi	+ rhi	- rhi	+ rhi	- rhi	+ rhi	- rhi	+ rhi
inoculation rate (%) upon harvest		70	85	63	63	89	69	17	57
ARA	mean	2.05	1.98	0.379	1.08	1.05	1.23	0.031	0.688
(μmol C_2H_4 h^{-1} g^{-1} f. wt)	SD	2.41	2.61	0.235	1.11	1.77	1.49	0.054	1.12
ARA	mean	0.153	0.462	0.019	0.144	0.066	0.212	0.003	0.057
(μmol C_2H_4 $plant^{-1}$)	SD	0.138	0.921	0.010	0.254	0.065	0.288	0.005	0.125
nodule f. wt (g)	mean	0.100	0.242	0.088	0.114	0.140	0.145	0.036	0.063
	SD	0.107	0.383	0.068	0.131	0.143	0.153	0.056	0.089
plant biomass (g d. wt)									
root	mean	0.840	1.06	0.886	0.808	1.11	1.10	0.578	1.26
	SD	0.904	0.65	0.537	0.401	0.67	0.74	0.310	1.38
stem	mean	0.689	1.03	0.753	0.571	0.552	0.792	0.488	1.09
	SD	0.908	0.72	0.436	0.217	0.214	0.466	0.236	1.18
leaf	mean	0.902	1.30	0.960	0.825	0.121	0.245	0.038	0.182
	SD	1.064	0.93	0.809	0.372	0.061	0.224	0.024	0.158
total	mean	2.43	3.39	2.60	2.21	1.78	2.13	1.10	2.53
	SD	2.85	2.21	1.67	0.948	0.85	1.24	0.52	2.59

Table 6.2 Correlations between legume biomass (g f. wt) and 1-h ARA (μmol C_2H_4 h^{-1} g^{-1} f. wt), after six-month of growth at landfill sites. - rhi = seedlings were rhiozobia-free when transplanted to sites; + rhi = seedlings were inoculated artificially when transplanted to sites; $P = 0.05$.

			root	stem	leaf	nodule	ARA f. wt^{-1}
Junk Bay							
Acacia confusa	- rhi	stem	0.976				
		leaf	0.988	0.978			
		nodule	0.753	0.691	0.776		
		ARA f. wt^{-1}	-0.174	-0.170	-0.150	-0.237	
		ARA $plant^{-1}$	0.705	0.667	0.742	0.905	0.180
	+ rhi	stem	0.957				
		leaf	0.768	0.874			
		nodule	0.733	0.821	0.899		
		ARA f. wt^{-1}	-0.203	-0.163	-0.169	-0.065	
		ARA $plant^{-1}$	0.725	0.815	0.853	0.976	0.042
Leucaena leucocephala	- rhi	stem	0.745				
		leaf	0.098	-0.247			
		nodule	0.790	0.508	0.363		
		ARA f. wt^{-1}	-0.474	-0.149	-0.262	-0.369	
		ARA $plant^{-1}$	-0.304	-0.210	0.171	0.200	0.688
	+ rhi	stem	0.498				
		leaf	0.789	0.530			
		nodule	0.833	0.087	0.692		
		ARA f. wt^{-1}	0.253	0.242	0.307	0.168	
		ARA $plant^{-1}$	0.692	0.203	0.529	0.650	0.807
Shuen Wan							
Acacia confusa	- rhi	stem	0.945				
		leaf	0.811	0.621			
		nodule	-0.269	0.033	-0.558		
		ARA f. wt^{-1}	0.152	-0.131	0.295	-0.810	
		ARA $plant^{-1}$	-0.054	0.181	-0.159	0.880	-0.835
	+ rhi	stem	0.891				
		leaf	0.860	0.956			
		nodule	0.801	0.607	0.751		
		ARA f. wt^{-1}	0.944	0.916	0.797	0.560	
		ARA $plant^{-1}$	0.894	0.747	0.850	0.981	0.702
Leucaena leucocephala	- rhi	stem	0.897				
		leaf	0.471	0.812			
		nodule	0.981	0.966	0.632		
		ARA f. wt^{-1}	0.980	0.967	0.637	1.000	
		ARA $plant^{-1}$	0.980	0.967	0.637	1.000	1.000
	+ rhi	stem	0.973				
		leaf	0.743	0.585			
		nodule	0.323	0.217	0.715		
		ARA f. wt^{-1}	-0.701	-0.770	-0.356	0.004	
		ARA $plant^{-1}$	-0.449	-0.446	-0.089	0.625	0.469

CHAPTER 7
DISCUSSION

7.1 Overview

The results of the field survey provided evidence for the abundant growth of leguminous trees on completed and on-going landfill sites in Hong Kong (Chapter 3). It also indicated that the presence of landfill gas in landfill topsoil was one of the limiting factors for the growth of trees. The effects of landfill gas on the growth and N_2 fixation of two rhizobia-legume systems (Acacia confusa, Leucaena leucocephala) were assayed under laboratory and greenhouse conditions (Chapter 4). In addition to the influence of landfill gas, the effect of landfill leachate on the symbiotic systems was presented in Chapter 5. To verify the results of the laboratory experiments, a field trial was conducted on landfill sites (Chapter 6). The overall results provided evidence for the presence of free rhizobia in landfill topsoil. They infected the legumes and formed effective nodules fixing N_2.

7.2 Soil conditions and tree growth on landfill site

The results of the field survey provided evidence that the landfill sites were unique habitats for tree growth, especially with respect to the gaseous composition of the soil. In the 13 surveyed sites, the O_2 and CO_2 concentrations at 35 to 50 cm depth of landfill topsoil ranged from 10.9 to 20.9% and 0.03 to 17.4%, respectively (Table 3.1). In contrast to complete landfill sites in temperate cities, which are unstable in gas production for at least five years (Dobson & Moffat, 1993), the field survey results provided evidence that the landfill gas levels in the topsoil of subtropical sites would decline rapidly within five years. The landfill gas levels were generally high at new or on-going sites, indicated by the low concentrations of O_2 (10.9 to 16.8%), high concentrations of CO_2 (3.76 to 11.29%) and high concentrations of CH_4 (4.18 to 17.5%) in Junk Bay Stage I, Junk Bay Stage II/III and Shuen Wan (all were on-going sites). Low levels of landfill gas were detected in the three sites closed for 6 to 10 years (Siu Lang Shui, Ma Yau Tong Central and Jordan Valley: O_2 > 19.8%, CO_2 <

0.53%, $CH_4 < 0.01\%$). Therefore, gas problem for woodland establishment in subtropical landfill sites occurs mainly in the first five years after site closure and in sites with similar capping as the landfill sites in Hong Kong.

The topsoil of most sites was acidic (8 of 13 sites < 6.0, minimum = 4.34) and therefore generally considered to be unsuitable for woodland establishment (Yu & Dong, 1990). The bulk density at eight sites was above 1.4 g cm^{-3}, which might suppress the root penetration of common plants (1.123). The N content in landfill soil was generally low, except for Gin Drinkers' Bay and Shuen Wan, which had a total extractable N content ($NH_4^+ + NO_2^- + NO_3^-$) > 1 mg N 100 g^{-1} (d. wt), the total extractable N content in all other sites were < 1 mg N 100 g^{-1} (d. wt). Such a low soil N content is generally considered deficient for supporting the normal growth of trees to establish a woodland (Song & Xu, 1990; Zhu, 1990). The landfill sites in Hong Kong were designed as a dry storage place (1.11); their final cover was properly levelled and compacted by steel wheel compactors and surplus rain water was collected by ditches. Therefore, waterlogging could only be observed in certain edge areas (3.3). This capping design caused a low moisture content in topsoil (7.5% (the lowest, at Shuen Wan) to 16.8% (the highest, at Ma Yau Tong Central)). The exceptionally high soil moisture in Ma Tso Lung (21.5%) was caused by the piling of sawdust on the site which prevented evaporation (3.3). Therefore, the results of soil properties and tree cover on this site were not suited for comparison with other sites.

Soil conditions at a landfill site depended highly on the civil engineering design and human activities. For example, the high landfill gas level at the old Ma Tso Lung Landfill (CO_2 = 17.4%, CH_4 = 28.2%, completed 15 years) was presumably due to its gas extraction system being primitive and the piling of sawdust on the site prevented gas diffusion from the soil surface to ambient. In contrast, the installation of a gas abstraction network in Sai Tso Wan Landfill (1.132) had vented effectively the landfill gas and caused a low gas level in the topsoil (CO_2 = 0.47%, Table 3.1).

Among all the soil conditions, the coefficient of correlation between the CO_2 and O_2 concentrations was one of the highest ($|r| = -0.975$, Table 3.5). As the O_2 in topsoil was displaced by the landfill gas, the correlation between the gases should be high. Similarly, high coefficients of correlation between the concentrations of CO_2, CH_4, O_2 and N_2 were found.

Although the tree cover on the 13 sites ranged from totally bare (Junk Bay Stage II/III) to 90% (Siu Lang Shui) (Table 3.6), the overall results indicated that the establishment of woodland on a subtropical landfill is possible. The total area of all 13 landfill sites was 268.1 ha and 136.1 ha was cemented (e.g. access roads, parking space) or on-going areas for waste dumping (Table 3.7). The remaining 132.0 ha was landscaped and allowed plant growth. However, only 34.1 ha of land was covered by trees. Therefore, 72.9% of the total landscaped land was bare of tree. Generally, the tree cover on new or on-going sites was low (e.g. Junk Bay Stage I, 10%; Junk Bay Stage II/III, 0%; Pillar Point, 20%; Shuen Wan, 1%), whereas the tree cover on old sites was high (e.g. Gin Drinkers' Bay, 50%; Siu Lang Shui, 90%). However, some of the old sites were also relatively bare. For example, the tree cover on Jordan Valley, Ma Yau Tong West and Ngau Tam Mei (all completed more than 5 years) were 5, 5, < 0.1%, respectively.

Correlation analyses were conducted to identify the key limiting factor(s) on landfill sites which affect the tree cover (Table 3.5). The results provided evidence that the presence of landfill gas in topsoil was the key limiting factor affecting tree cover, indicated by the relatively higher negative coefficient of correlation between the CO_2 concentrations and tree cover ($r = -0.415$; $P = 0.05$) and between the O_2 concentration and tree cover ($r = 0.44$; $P = 0.05$). The effect of landfill gas was pronounced at most of the on-going sites. The tree covers on the on-going sites on Junk Bay Stage I, Stage II/III and Shuen Wan were < 10%, perhaps due to the high landfill gas levels in the topsoil ($CO_2 > 3.76\%$).

In addition to landfill gas, there are quite a lot of other unique features on a landfill site that would affect the overall performance and growth of trees. For example, the presence of an impermeable cap on the Pillar Point Landfill (bulk density = 1.42 g cm^{-3}) (Table 3.2) caused a low gas level in the soil ($CO_2 = 0.05\%$) and only 10% of the landscaped land on the site had been covered by trees. However, it is hard to identify the key limiting factors at some of the sites. For example, the relative high geological setting of the Jordan Valley Landfill (3.4) might caused a lower water table on the site and the lack of water supply would be one of the major limiting factors caused the poor tree growth (tree cover = 5% of land), especially on the upper platform (nearly totally bare). Although this hypothesis had been partially reviewed by the relatively low soil moisture (12.6%) measured at the site, it could only be proved when a long-term monitoring of soil properties program had been applied.

Landscaping work can compensate partially for the above mentioned adverse factors. In Junk Bay Stage I and in Pillar Point, their relatively high tree covers (both 10%) were presumably caused by the intensive tree planting work on sites, proved by the fixed pattern of tree growth along grid lines, while similar tree planting on Shuen Wan and Junk Bay Stage II/III was not seen.

Regardless of the above limiting factors, a total of 41 species of tree was found on the 13 sites. Of these, 10 were legumes and 31 were non-legumes (Table 3.6). In terms of tree cover, 22.3 ha of land was covered by legumes and 11.8 ha was covered by non-legumes. When the relative tree covers of legumes and non-legumes were compared, legumes became relatively abundant on landfill sites and occupied 65.4% of the total area which had a tree cover.

Within the ten species of legume, Acacia confusa and Leucaena leucocephala were mostly abundant, especially Acacia confusa. 55.1% of the total tree covered land were covered by this species. Presumably, most of the A. confusa were transplanted to sites by landscape contractors, indicated by their pattern of growth (3.4). However, self-seeded young seedlings were also common on most of the sites. Although the high value of cover by A. confusa was partially man-made, the survey results provided evidence that this species was quite suited for growth on completed landfill sites. It survived and was abundant on most completed landfill sites where post-transplantation care was generally limited.

Leucaena leucocephala (another Mimosaceae) was the second most abundant legume on the sites. This overall relative tree cover was 5.3%. The random scattering on sites, sometimes growing in cracks of rocks, the unpruned growth and high variability in height showed that the trees were self-seeded on the sites.

7.3 Effects of landfill gas

The field survey indicated legumes were relatively abundant at completed landfill site and landfill gas was one of the major limiting factors affecting tree cover (Chapter 3). Although landfill gas may include a wide range of gases and some of which are to be highly toxic to plants, their concentrations are generally very low (1.121). To study the influence of landfill gas on N_2 fixation, bioassays were conducted on the two legumes mostly abundant on complete sites (Acacia confusa and Leucaena leucocephala) and confined on three major components of landfill gas: O_2, CO_2, CH_4. The influence of these gases on the rhizobia-legume systems was investigated by short-term (1-h) and long-term (4-week) assays (Chapter 4).

The influence of O_2 supply in the rhizosphere for nodular N_2 fixation was observed in the 1-h short-term bioassay (4.2). Maximum ARA was found at 20.0% O_2. When the O_2 concentrations were lowered to 16 and 8%, C_2H_2 reduction started at low levels and increased slightly. The results provided indirect evidence for the presence of a regulatory mechanism in bacteroids to supply O_2 for the bacteroids under sub-ambient O_2 concentrations at $\geq$ 8% (1.311). The decline in ARA on the two legumes under sub-ambient O_2 concentrations was similar to the responses of some legume herbs under O_2 depleted conditions. All legume nodules have a common regulatory mechanism for O_2 supply, so their responses to changes in external O_2 supply should be similar. However, the regulatory mechanism has its limitation. When the external O_2 concentrations decreased below 4%, nodules of the two legumes could no longer fix N_2, indicated by the significant reduction of ARA at $\leq$ 4% ($P < 0.05$) (Fig. 4.4).

For both legumes, the extremely low level of ARA at $\leq$ 4% O_2 being determined might be caused by the small amount of residual O_2 present in the nodules which was available for the bacteroids. The cause of the sharp decline in the nodular ARA of Leucaena leucocephala at 16% was unknown. It might be caused by the less energy supply from the host to the nodulated roots as the shoot was removed before ARA. Although the short-term assay was 1-h, a complete experiment of a single series of a gas and with three replicates for each concentration lasted for three consecutive days and involved more than 20 legume seedlings. Variations in the host physiological status and the testing time on a day might cause the high deviation on the ARA (1.322).

The field survey provided information that the O_2 concentration within the upper 35 to 50 cm of topsoil at the 13 landfill sites was generally > 10% (Table 3.1). From the results of the 1-h ARA bioassay (4.2), N_2 fixation of the nodules under such levels should be reduced by less than 70%, in comparison with ARA under ambient O_2 conditions (Figs 4.4, 4.5).

Although stimulatory effects of low concentrations of CO_2 (< 0.5%) in the rhizosphere have been reported on some rhizobia-legume systems (1.312), the influence of CO_2 at the higher concentrations sometimes occurring in landfill topsoil appears to be lacking. The results of the short-term assay provided evidence that when the CO_2 concentrations were elevated to the range of 10 to 20%, C_2H_2 reduction of the legume nodules would be induced for about 30 min (Fig. 4.2). The duration and degree of induction depended on the concentrations of CO_2 and there was variation among species. Comparison of the responses of the two legumes showed that the stimulation of CO_2 on ARA in the Leucaena leucocephala was higher than in the Acacia confusa, indicated by the 15-min interval time-course C_2H_2 reduction (Fig. 4.2) and in the overall 1-h ARA (Figs 4.4, 4.5). After the short induction period, the presence of CO_2 inhibited the C_2H_2 reduction of the nodules. For both species, the inhibitory effect of CO_2 was pronounced when it was elevated to > 30%, as a net increase in C_2H_2 reduction at these levels was hardly detectable after the first 15 min (Fig. 4.2). There should be a time-lapse for the CO_2 to express its effect on nodules and then being detected by the gas chromatography after the C_2H_2 was injected into the assay systems; as the nodule cortex is a good barrier for gas (1.311). In order to know more about the exact influence of CO_2 on N_2 fixation of rhizobia-legume system, more experimental data are needed on shorter intervals of C_2H_2 reduction and with more dilution points at < 20% CO_2. However, the overall results of the two legumes indicated no significant reduction ($P > 0.05$) was detected on their 1-h ARA at 10 - 20% CO_2.

A maximum concentration of 64% CO_2 in landfill soil has been reported for UK (Table 1.1). However, the concentration of CO_2 at a certain point of topsoil of a site is generally less than the maximum concentration detected detected on the same site (1.121). Moreover, the concentration of CO_2 in landfill topsoil is generally in proportion to the depth of the sampling point (Department of the Environment, 1994). The highest CO_2 concentration detected at 35 to 50 cm of topsoil at the 13 landfill sites was about 10% (3.3). However, most nodules of the two legumes were relatively abundant on roots at 5 to 20 cm deep of topsoil (6.3), where the CO_2 concentration was very likely lower than at the depth of 35 to 50 cm. The 1-h ARA assay provided evidence that the nodules of both legumes fixed N_2 at 10% CO_2. Therefore, it is very likely that nodules at landfill topsoil can fix N_2 under landfill topsoil CO_2 conditions. When the CO_2 concentration in soil was elevated for a short period (e.g. < 1 h) and was maintained at about 20%, nodular ARA would be induced. However, the nodular ARA would be inhibited when the CO_2 concentrations in topsoil were elevated to > 20% and lasted for > 1 h.

The 1-h assay showed CH_4 had neither stimulatory nor inhibitory effects on the ARA of root nodules (Fig 4.3). As a safety measure, the concentrations of CH_4 of a site should be monitored closely by landfill operators. Their concentrations on a site can also be considered as an indirect indication of the concentrations of O_2 and CO_2 in soil, as their concentrations are highly correlated (Table 3.5).

The results of the four-week long-term assay provided evidence for the sub-lethal inhibitory effects of landfill gas when legumes were exposed to the gases for an extended period (4.32). Despite the difference in the duration of assay period, intact plant samples were used in the four-week assay, whereas nodulated roots were used in the 1-h short-term assay. Therefore, the four-week long term test studied the influence of the gases on the whole rhizobia-legume system.

The adverse effect of CO_2 was indicated by the significant reduction ($P < 0.05$) of nodular ARA of Leucaena leucocephala after the seedlings were fumigated with CO_2 for four weeks. The inhibition of the growth of plants by CO_2 was expected, as such inhibition has been reported for various plants and by various authors (1.312). However, in the presence of the CO_2, rhizobia assisted the growth of the host legumes, indicated by the 8.8% higher biomass in the infected plants in comparison with the rhizobia-free seedlings. In both Acacia confusa and Leucaena leucocephala, the fumigation of landfill gas caused a similar influence on the rhizobia-legume systems as caused by the CO_2 treatment and the growth and N_2-fixing activity of the nodules were suppressed. However, under the influence of landfill gas, rhizobia-infected legume seedlings had a higher biomass (26.8 - 56.0%) than rhizobia-free seedlings.

The overall results of the long-term assay indicated that leguminous nodules might overcome the adverse gaseous condition of landfill topsoil which might have high CO_2 and low O_2 concentrations. Similar tolerance of cowpea and soybean nodules at extremes of sub- and supra-ambient O_2 concentrations (1 - 80%) was reported by Dakora et al. (1991); neither the proportional composition of leghaemoglobin nor its oxidation state was affected after 28 days.

7.4 Effects of landfill leachate

Localized leachate contamination is a common problem of woodland establishment on completed landfill sites (1.122). Once the top liner is punctured and a leachate leak occurs, it is difficult to reform the liner and a high lethal rate of trees is expected in the leachate contaminated zone. Therefore, there is a need to seek tolerant species to vegetate the problem areas. In addition to using highly tolerant species as a remedial measure, leachate recirculation is a kind of leachate treatment. The influence of leachate addition/contamination on soil was investigated in Chapter 5 on four species of tree (2 legumes, 2 non-legumes) and with special emphasis on the role of rhizobial infection.

The results of the leachate bioassay indicated that prolonged leachate irrigation at a high dose is lethal for both legumes and non-legumes. The net respiration rates of leaves at the highest concentration of leachate indicated that normal photosynthesis had ceased (Fig. 5.1). Furthermore, the reduction in their total biomass at the highest concentration of leachate provided evidence for their retarded growth (Figs 5.2 to 5.5).

For all the four species of tree, 10 ml of 18.0 and 4.56% leachate d^{-1} caused higher accumulated plant biomass (Fig. 5.2 to 5.5). At the lowest leachate concentration (0.58%), there was a deficiency in mineral supply for the legume seedlings, as extractable minerals from the mixture of sandy soil and vermiculite were low (Table 5.2). Seedlings at such low concentration of leachate produced less biomass (Fig. 5.2 to 5.5) and ARA (Fig. 5.6).

The N_2 fixation of the two legumes was suppressed markedly under the influence of leachate addition as ARA was non-detectable in most seedlings (Fig. 5.6). The presence of high levels of compound N in the leachate (Table 5.1) is likely to be one of the inhibitory factors which affect nodular N_2-fixation (1.313). ARA was unlikely to have been inhibited by toxic metals because there was no detectable difference in the concentrations of Ni, Cu, Zn or Cd (1.335) after leachate addition in comparison to the control (Figs 5.10 to 5.13). However, the Ni and Cd concentrations in the plant samples (10 - 40 and 1 - 8 $\mu g\ g^{-1}$ d. wt), after leachate treatment and in the control, were relatively high compared with the typical range for these elements in plant tissue (0.5 - 5 and 0.01 to 0.3 $\mu g\ g^{-1}$ d. wt) (Allen, 1989b). The cause of the increases in these elements can only be explained when more experiments on these legumes growing on metal-contaminated soil are tested and compared.

Although the N_2-fixing activity of the nodules in both legumes was suppressed, the infected seedlings generally showed higher total biomass (5 to 60%) than rhizobia-free seedlings at the same concentration of leachate (Figs. 5.2, 5.3). Moreover, the presence of rhizobial infection prevented the excessive accumulation of N in the plants from leachate contaminated soil (Fig. 5.7). Regulation of N uptake from soil to legumes by root nodules was reported (Hunt & Layzell, 1993; Malik et al, 1987) but the exact control mechanism is unclear.

The performances of legumes and non-legumes was compared and seedlings of two non-leguminous plants were treated similarly to the legumes (Cinnamomum burmanii, Tristania conferta). The non-legumes were sensitive to leachate and started to die far before the legumes gave any symptoms of abnormality.

Both rhizobia-free and infected legume seedlings survived the highest dose of leachate four times longer than the two non-legumes. This indicated that legumes are more tolerant than non-legumes to leachate contamination, regardless of rhizobial infection. The higher N demand and N content in their tissue (Bressani & Elias, 1980), as a basic characteristics of legumes plants, are some of the superior features for them to adapt on leachate-contaminated-soil .

7.5 Role of rhizobia-legume symbiotic N_2 fixation under landfill conditions

The growth of the two legumes under actual landfill conditions was investigated by transplanting rhizobia-free and pre-inoculated seedlings to landfill sites with high landfill gas in their topsoil (Chapter 6). The results provided evidence that there were free rhizobia at the sites to infect the legumes, as 17 to 89% of rhizobia-free seedlings became infected within six months (Table 6.1). The nodules formed under landfill site conditions were effective in fixing N_2, as shown by the results of the ARA studies. Therefore, when legumes are used to revegetate completed landfill sites, it is probably unnecessary to pre-inoculate the seedlings to be transplantd to the sites. There should be enough free-rhizobia to infect the host. However, such high infection rates of the two legumes are likely to be related to a high abundance of their mature trees on the sites. If legume species rarely seeded naturally or are rarely planted in landscaping work, the presence of an adequate free rhizobial population in soil would be uncertain, especially as rhizobia are host-specific (1.21).

The field trial provided evidence that rhizobial infection assisted the growth of the legumes under landfill conditions, indicated by the higher plant biomass in the pre-inoculated seedlings than seedlings which were rhizobia-free when transplanted to the sites (Table 6.1). The results of the field trail also verified the results of the bioassays of legumes and their associate nodules at different concentrations of O_2 and CO_2 (Chapters 4 and 5) that rhizobia can survive, fix N_2 and assisted the general growth (in term of biomass) of legume plants at landfill gaseous conditions.

The coefficient of correlation between ARA and plant biomass in the pre-inoculated seedlings was generally higher than in the seedlings in the rhizobia-free group. This indicated that the host and symbiont relationship was better established in the pre-inoculated group than in the rhizobia-free group. Such differences also indicated that a period longer than six months was required for the legumes to be infected and to form mature and effective nodules on the host.

The coefficient of correlation between the leaf and nodule biomass (> 0.6, P = 0.05, except Acacia confusa at Shuen Wan) was generally higher than the coefficient of correlation between the leaf biomass and ARA. Samples having higher plant and nodule biomass might not necessary to have a higher ARA because the N_2 fixation of nodules depended highly on external factors, e.g. temperature and intensity of solar radiation (1.32) and seedlings from different sites were not harvested on the same date.

7.6 Concluding remarks

The results of Chapter 3 provided evidence for the presence of adverse factors in landfill site for the growth of rhizobia-legume tree system. The positive role and function of rhizobial infection in legume trees for woodland establishment on completed sites were assessed in Chapters 4 to 6. The topsoil properties of the landfill environment are unique (3.3), their influence on legume shrubs and legume herbs is an important research topic and one with a high practical value. Legume shrubs and herbs should also be some of the tolerant pioneer plants to become established on completed sites; if they bear similar responses to landfill stress as the two woody legumes being assessed.

Only ten-month old legume seedlings were assessed and their performance at the sites was investigated for half a year. The root penetration of the seedlings in the topsoil was limited within this short project period and the inoculation and N_2 fixation of nodules at deep root system close to the sub-soil was not quantified. Further information on the influence of landfill factors on mature legume trees can only become available when their root growth pattern and inoculation processes have been studied and the N_2 fixation of nodules tested.

In addition to the relationship between rhizobia and legume, the function of mycorrhizal fungi on legumes and under landfill conditions is uncertain. Generally, the presence of mycorrhizae may assist legumes to take up P and other nutrients from soil and nodulation is poor when mycorrhizae are absent from the rhizosphere (Munns & Mosse, 1980).

Subtropical places, like Hong Kong, are especially well suited for the growth of legume plants (1.21). In addition to Acacia confusa and Leucaena leucocephala, other legumes potentially suitable for woodland establishment on completed landfill sites should be tested. Studies are also needed to establish the possible importance of other N_2-fixers, such as some Casuarina species (Baker & Mullin, 1992; Becking, 1992). Another plant with requires study at the sites is Sesbania. S. cochinensis was common in most of the 13 sites, although its growth and tree cover were not reported in Chapter 3 as it is not a perennial. Stem nodules in Sesbania spp. and in some other trees are well known to be active in N_2 fixation (Evans & Burris, 1992).

SUMMARY

1) An ecological survey was conducted on the 13 completed and existing landfill sites in Hong Kong. Leguminous trees were abundant on most sites, indicating that they were more suited to growth under landfill conditions than non-legumes.

2) The ARA of nodules of two species of legume, Acacia confusa and Leucaena leucocephala, was tested at different partial pressure of O_2, CO_2 and CH_4. The responses of their nodules towards the gases were similar. Under sub-atmospheric partial pressure of O_2, the nodular ARA was reduced in proportion to the concentrations of O_2. In the presence 10 to 50% CO_2, the ARA of the nodules was suppressed in proportion to the concentrations of CO_2. However, ARA of neither species ceased within the 1-h assay period in the presence of the CO_2, even at the highest concentration of 50%. Methane (10 to 50 %) did not affect the ARA within 1 h.

3) The results of a four-week long-term simulated landfill gas treatment proved that landfill gas suppressed the ARA of root nodules of both Acacia confusa and Leucaena leucocephala. However, the gas itself was neither lethal to the rhizobia nor to the host.

4) A greenhouse experiment was conducted to investigate the effects of landfill leachate on the growth of legumes and non-leguminous trees. The results indicated that Acacia confusa and Leucaena leucocephala, with or without rhizobial infection, were far more tolerant than the non-legumes Cinnamomum burmanii and Tristania conferta. The legumes survived five months at the highest leachate concentration (i.e. 10 ml 73% leachate daily per seedling), while the non-legumes tolerated the same concentration for one month only. The longer survival period of the rhizobia-free legume seedlings provided evidence that legumes are tolerant to leachate whether or not they possess nodules in their root system.

5) Irrigation with optimal levels of leachate (i.e. 1.14 to 18.0% generally, with variation between species) benefited the growth of all the four species of tree, indicated by the increases in their biomass. However, leachate at high concentrations (i.e. at 73.0%) suppressed the growth and became lethal for the plants.

6) The effect on N_2 fixation of legume nodules was assayed under the influence of leachate. Landfill leachate suppressed (24 to 100%) the ARA of the two nodules and the high compound N in leachate is suspected to be the active ingredient for the effect. However, the growth of rhizobia-infected seedlings was generally better than rhizobia-free seedlings, indicated by their biomass after treatment.

7) The higher total N levels in the leaves and stems of rhizobia-free seedlings of both species of legume after the leachate treatment indicated rhizobial infection assisted the legumes in the control of compound N assimilation from soil and avoided the accumulation of excessive N in the plant tissue. Higher total P content was found in the infected Acacia confusa seedlings after the leachate treatment, in comparison with the rhizobia-free seedlings.

8) Rhizobia-free and infected seedlings of Acacia confusa and Leucaena leucocephala were transplanted to two landfill sites to test for their performance under actual landfill conditions. Most (17 to 89% depended on species and sites) of the rhizobia-free seedlings became inoculated within six months. The results indicated that under landfill conditions, there were free rhizobia in landfill soil to infect legumes. Moreover, the rhizobia formed effective nodules on the host to fix N_2, determined in terms of ARA.

9) The harvestable biomass of pre-inoculated legume seedlings after six months growth at the landfill sites was 20 to 130% higher than the harvestable biomass of seedlings which were rhizobia-free when transplanted to the sites. These results are evidence that

nodules assisted both species of legume to grow better, in terms of biomass, under actual landfill conditions.

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